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DETERMINATION OF NORMAL TEMPERATURES BY MEANS OF THE EQUATION OF THE SEASONAL TEMPERATURE VARIATION AND A MODIFIED THERMOGRAPH RECORD

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In the fall of 1918 Logan, Utah, was paving some of its streets. Because of the labor shortage, the work had been delayed and it was feared that freezing weather would set in before the work could be completed. The city engineer asked the writers what temperature Logan would be likely to experience during the third week of November between 4 and 6 p. m. They could not tell him.

In attempting to answer this question the writers were attracted to a study of seasonal temperature change and also to the change in temperature as the day advances. These temperature changes occur rather gradually and regularly. It becomes slowly warmer as the day advances; and after the setting of the sun the air cools, until just before sunrise, when it begins to warm again. This is repeated each day. The mean daily temperature gradually rises as the summer approaches, reaches a maximum, begins to fall, and reaches a minimum in the winter. The writers undertook to determine, from the temperature observations of the Weather Bureau, the law connecting the temperature and the time, for the purpose of determining the probable temperature at a particular place for a particular day and a certain hour of that day.

LITERATURE

Lambert, Weilenmann, Maurer, Lamont, Trabert, and Angot worked on the problem of the diurnal change in temperature in an attempt to give a mathematical expression for the temperature change in which the constants of the equation had a physical significance.[2] The upper part,

[1] The authors wish to thank Dr. Willard Gardner, Associate Physicist at the Utah Agricultural College, for helpful suggestions on this problem.

[2] PUTNAM, J. M. PRESENT STATUS OF OUR KNOWLEDGE OF THE CAUSES OF THE DIURNAL CHANGES IN TEMPERATURE, PRESSURE, AND WIND. *In* Mo. Weather Rev., v. 42, no. 12, p. 655-665. 1914. Notes and references, p. 662-665.

Discusses in detail articles by Lambert, Weilenmann, Maurer, Lamont, Trabert, and Angot. The original of these articles not available to the author of this paper.

or daytime period, has so many physical factors involved that the problem was very difficult. The night interval, or lower part of the curve, they found to be logarithmic, the constants of the equation having a physical significance. The equation of the upper part of the curve, the equation of the lower part of the curve, and a combined equation of the two—partly empirical—have been worked out. Occasionally a section of the country experiences a warm winter or a cold summer.[1] These temperature courses have been studied by Gawthrop.[2]

ANNUAL TEMPERATURE CHANGE

The seasonal change in temperature is due to the fact that the earth is moving around the sun in an elliptical orbit with a variable speed, its axis of rotation making an angle of 23½° with the plane in which it revolves. The 24-hour temperature variation is due to the rotation of the earth on its axis.

If the rate of emission of heat by the sun were to remain constant and if the rate of rotation and of translation of the earth be the same each year—the path of the earth around the sun being the same each year—and if the diathermancy of the atmosphere were to remain constant with no evaporation or condensation, the sequence of temperatures of one year would be very nearly exactly that of any other year, and the thermograph record, for April 1, for example, would be the same every year. On cloudless days this is nearly realized. The foregoing conditions are nearly realized except for the diathermancy of the atmosphere, which varies because of evaporation and cloud formation. It is because of the passage of cyclones and anticyclones—storm and fair weather areas—across a section with the accompanying rain or snow, the dissolving of clouds, or evaporation of rain, that we have temperature departures from normal.

In order to determine the normal change in temperature with the time over the entire year, it would be necessary to get the mean temperature for every hour of the year for enough years to eliminate the irregularities due to storms, and then make a plot of these 8,760 (24 × 365) hourly temperatures and determine the equation of the curve. This is possible, but it is obviously a very tedious operation. The same result may be obtained much more easily by dividing the problem and considering the seasonal temperature change and the daily change separately. In other words, it will be shown how to determine the mean daily temperature, and this value will be multiplied by a certain percentage in order to get the value for a certain hour of the day.

However, the arid West, comprising the section between the Rocky and Sierra Nevada Mountains, has only about 10 inches of rainfall and little cloudiness, a humidity of but 50 per cent, and 300 days of the year

[1] PENTER, J. M. OP. CIT.

[2] GAWTHROP, HENRY TEMPERATURE COURSES. *In* Mo. Weather Rev., v. 35, no. 12, p. 576-578, 1 fig. 1907.

without rain. In addition, fully one-fourth of the land area of the earth is equally dry. The departures from the normal temperature, therefore, are least for these areas; and the method to be developed in this paper is most useful for them in predicting actual temperatures.

Figure 1 represents the mean monthly temperatures for several widely separated cities of the United States and shows how the temperature changes as the seasons advance. The curves are somewhat alike. For Seattle and San Francisco they are flatter than for the others, showing that the difference in temperature between summer and winter is slight. These cities are said to have an equable or oceanic climate because of their relative position to the ocean with its high heat capacity and the prevailing westerly winds. It is probable that for cities with the same mean annual temperature and with the same difference in temperature

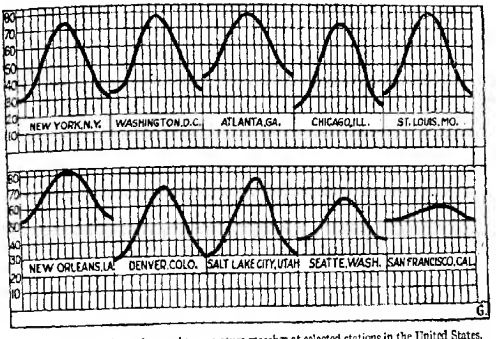

FIG. 1.—Synoptic chart of annual temperature marches at selected stations in the United States.

between summer and winter the curves would be just alike. For cities with the same annual variation the curves would have the same slope.

Figure 2 shows the change in temperature with the season in Utah and represents the mean monthly temperatures for the State.

Any single-valued periodic function can be expressed by an infinite trigonometric series, or Fourier series, of the form

$$T = a + b \sin x + c \sin 2x + d \sin 3x + \ldots\ldots$$
$$+ e \cos x + f \cos 2x + g \cos 3x + \ldots\ldots\ldots (1);$$

or if it is desirable to express it in terms of the cosine only, it takes the following form:

$$T = a + b \cos (\theta - c) + d \cos 2 (\theta - e) + f \cos 3 (\theta - g) + \ldots (2),$$

the contents a, b, c, d, etc., determining the shape of the particular curve. The method is well known to mathematicians and is explained in the larger texts on calculus under the head of Fourier's series.

The following is the equation of the curve of figure 2, which represents the seasonal temperature change for Utah:

$$T=48.5-20.91\cos\theta-1.28\cos 2\theta-0.67\cos 3\theta+\ldots\ldots$$
$$-7.57\sin\theta+2.38\sin 2\theta-0.83\sin 3\theta+\ldots\ldots\ldots\ldots \quad (3),$$

or in terms of one trigonometric function only:

$$T=48.5-22.2\cos(\theta-19^\circ-54')-2.70\cos 2(\theta-149^\circ-5')$$
$$-1.03\cos 3(\theta-17^\circ-3')+\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4),$$

where T represents the temperature at the time θ. The first constant in each of these equations (48.5) is the mean annual temperature for the State, expressed in degrees Fahrenheit.

In the last two columns of Table I it is shown that for the 30 county seats of Utah the mean annual temperatures differ from the mean for

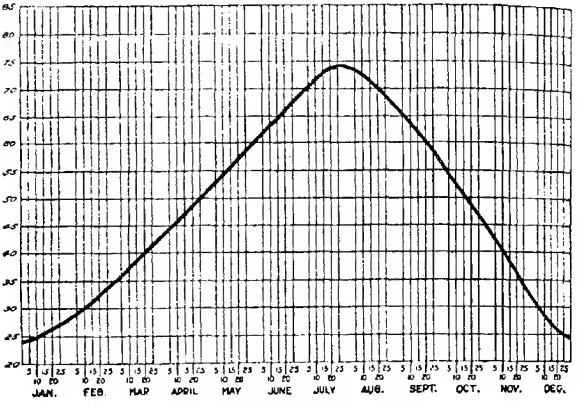

FIG. 2.—Mean monthly temperatures for Utah.

the State by from 0° to 10° F., with a mean departure of 4°, and that the mean annual range for these towns differs from the State range 0° to 9°, with a mean departure of 4°. The first constant in the equation simply moves the graph as a whole up or down the page without changing its shape. Inasmuch as the annual range in temperature—the difference in temperature between summer and winter—is nearly the same for all these county seats, making all their curves of approximately the same shape, the above equation can be used for any of Utah's towns or any other place that has the same annual range by replacing the first term by the mean annual temperature for the place in question.

To determine the mean daily temperature on April 1 for Salt Lake City, change the date to degrees by dividing the number of days that have elapsed since January 1 by 365 and multiply by 360, which gives

90, and use 90 for θ in the equation, and for the first term of the equation use the mean annual temperature for Salt Lake City (52° F.) instead of 48.5°, and by means of a table of cosines calculate the desired temperature from formula 4.

TABLE I.—*Mean monthly temperatures with daily and annual ranges (in degrees Fahrenheit)*

Station.	County.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Annual.	Mean daily range.	Mean annual range.
Beaver	Beaver	29	30	39	54	61	68	73	68	58	48	39	27	48	31	39
Castle Dale	Emery	19	26	38	46	54	63	69	68	59	47	37	23	46	34	50
Corrinne	Box Elder	24	30	40	50	59	70	78	74	65	51	37	28	51	34	50
Duchesne	Duchesne	19	22	35	46	52	61	68	67	58	46	33	19	44	29	54
Escalante	Garfield	26	33	42	48	55	65	69	68	60	50	40	29	49	30	52
Farmington	Davis	29	33	42	49	56	65	73	71	66	50	40	29	49	31	43
Fillmore	Millard	31	34	42	50	58	68	75	74	65	53	42	30	52	33	45
Ft. Duchesne	Uinta	14	18	34	46	54	62	68	67	58	45	33	19	43	33	55
Heber	Wasatch	21	24	34	44	52	59	66	66	55	45	35	22	44	33	46
Henefer	Summit	22	26	35	44	51	58	65	64	55	45	35	24	44	34	43
La Sal	San Juan	25	28	35	45	51	61	67	66	57	46	37	25	45	24	42
Levan	Juab	25	29	37	46	53	64	71	70	60	48	37	26	47	27	47
Loa	Wayne	25	24	37	41	49	59	66	63	53	41	31	22	42	35	41
Logan	Cache	24	27	36	48	54	63	72	71	61	50	38	26	47	22	47
Manti	Sanpete	25	28	33	46	55	65	69	68	59	48	37	25	47	30	45
Marysvale	Piute	28	31	36	45	53	60	66	65	58	47	37	25	46	34	41
Meadowville	Rich	22	23	29	42	49	57	65	63	54	44	33	24	42	....	44
Moab	Grand	29	36	43	55	64	72	78	75	66	54	41	30	53	31	50
Morgan	Morgan	23	27	36	46	52	59	66	66	56	46	36	25	45	31	43
Ogden	Weber	29	32	41	51	59	69	77	75	63	51	40	31	52	....	47
Parowan	Iron	29	32	39	47	55	64	71	67	60	49	39	29	49	30	41
Provo	Utah	27	31	40	48	57	64	72	70	60	49	39	26	49	31	46
Richfield	Sevier	27	32	40	48	55	63	70	68	59	45	38	28	48	35	47
Salt Lake City	Salt Lake	29	33	42	50	57	67	76	75	64	52	41	32	52	20	47
St. George	Washington	38	42	49	57	66	76	82	81	71	59	46	38	59	37	55
Tooele	Tooele	29	33	40	49	55	65	73	73	63	50	40	31	50	22	44
Woodruff	Rich	15	17	28	40	47	55	61	60	51	41	30	19	39	33	45

Most of the interior cities of the United States have an annual variation in temperature differing from that of Utah by not more than 10° F. (see fig. 1). Since it is this factor that largely determines the shape of the curve, approximate mean daily temperatures can be obtained for any of these cities by means of equation 4 by using the mean annual temperature for the particular city in place of the first term (48.5).

The series converges rather rapidly, the omission of the fourth term making an error of one-half of 1 per cent and the omission of the third and fourth terms making an error of 2 per cent.

DAILY TEMPERATURE CHANGE

The difference in temperature between day and night in Utah—that is, between the maximum and the minimum for the 24 hours—is about 30° F. in summer and only about 15° in winter. This is because the sun attains a greater altitude at noon in the summer and heat is being received from the sun more rapidly on unit area than in the winter time, which causes the temperature to rise faster and reach a higher value for the same time period. Cooling takes place faster at night in the

summer time than in winter because of the greater difference in temperature between earth and upper atmosphere in the summer. The daily temperature change curve, or thermograph record, is very much flatter in winter than in summer. Figure 3 clearly shows this fact. Because this curve for the same place is continually changing as the season progresses, no direct attempt was made to determine its equation.

Figure 4 shows thermograph records from four widely separated cities of the United States. It is to be observed that the hottest time of day is about 4 o'clock—it varies with the season—instead of noon, and the

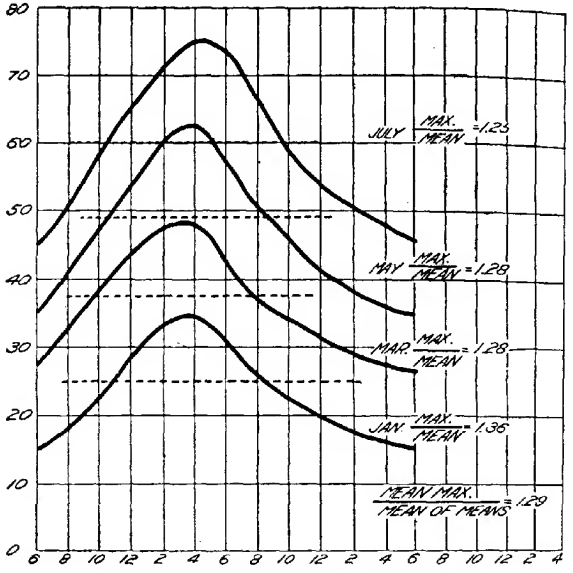

FIG. 3.—Average Utah thermograph records for various seasons.

coldest time is just before sunrise, instead of at midnight, as is sometimes thought. Heat is being received most rapidly at noon, but the temperature will continue to rise until the heat received and the heat lost per second are equal. As soon as the radiation rate exceeds the absorption rate the temperature will fall. Just after noon, even though the rate of absorption of heat is somewhat less than it was, yet it is still in excess of the radiation rate, and hence the net result is a rise in temperature. Inasmuch as this phenomenon is common to all localities, the thermograph records for clear days are very much alike in shape the country over. The convexity of the curve on the rising part or during the morning

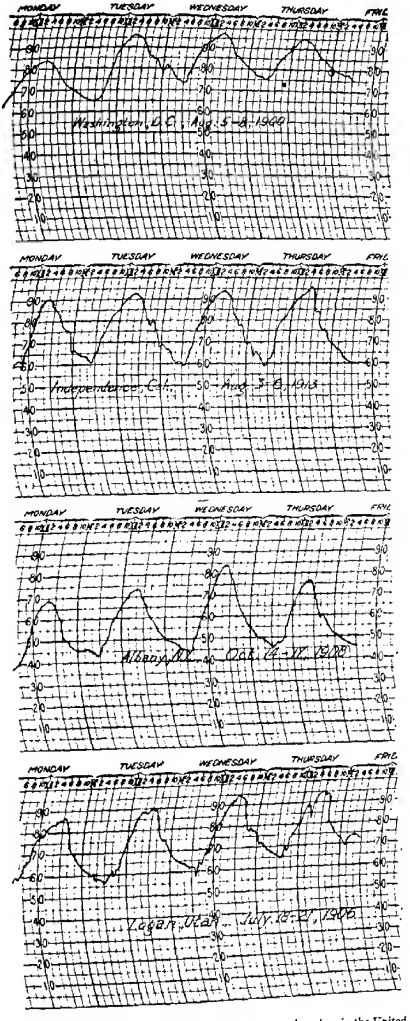

FIG. 4.—Actual thermograph records taken at widely separated stations in the United States.

hours and the concavity during the falling temperatures is mostly due to curvature of the vertical lines of the coordinate paper made necessary by the mechanism of the thermograph. The same temperature plotted on rectangular coordinate paper would make the curve approximately the shape of those of figure 1.

At first the writers thought that after having determined the mean daily temperature for the particular day they would determine the temperature for that particular hour by adding or subtracting a constant. The great change in the shape of the curve makes this method impracticable. However, it was found that if the temperature at a particular hour, such as the 3 a. m. value, is subtracted from the mean for the day and this answer divided by the mean for the day, the same answer will be obtained within less than 10 per cent no matter what day of the month or what month of the year is used. The variations are largely due to storms.

From a study of the temperature records of all the counties of Utah and for all months of the year the writers have determined the relation—expressed in percentage—of each hourly temperature to the mean daily temperature. These percentages of the mean for each of the 24 hours of the day are given in Table II. They come out practically constant for a particular hour of the 24, irrespective of the season, because as winter approaches and the mean daily temperature becomes lower the daily variation becomes less, the curve flattens (see fig. 3), and thus both values become less.

TABLE II.—*Relation of hourly temperatures to mean daily temperatures*

A. M.

Hour.	1	2	3	4	5	6	7	8	9	10	11	12
Per cent.....	85	82	79	76	73	71	72	73	77	87	97	108

P. M.

Hour.	1	2	3	4	5	6	7	8	9	10	11	12
Per cent.....	119	126	128	129	126	121	115	108	102	97	93	89

These percentages for the different hours are plotted in figure 5. The equation for this curve has been obtained by the method explained earlier and is as follows:

$$T = 97.33 - 9.8 \cos\theta + 0.88 \cos 2\theta - 0.52 \cos 3\theta - 23.26 \sin\theta + 3.52 \sin 2\theta - 1.42 \sin 3\theta + \ldots \quad (5),$$

or in terms of just one of the trinonometric functions:

$$T = 97.33 + 25.22 \cos(\theta - 67° - 10') + 3.71 \cos 2(\theta - 37° - 59') - 1.51 \cos 3(\theta - 23° - 16') + \ldots \quad (6).$$

This result will be accurate for normal temperatures, and the weather forecast will help one to predict the actual temperatures, because it is abnormally warm just before and cold just after the passage of a cyclone.

A less scientific method but one requiring only arithmetic and a knowledge of the mean monthly temperature for the place will now be given for the solution of this problem.

From figure 2 it is seen that the curve is approximately flat at the top from July 15 to August 15 and at the bottom from December 15 to January 15. To get the mean daily temperature for any particular day of one of these months simply use the mean value given for the month. For other months of the year the mean daily temperature changes by one-third of 1° F. a day.

Suppose it is desired to determine the temperature at Ogden on September 21 at 1 p. m. In Table I it is seen that the mean September temperature for Ogden is 63° F. Since the twenty-first is six days removed from the fifteenth, multiply ⅓° by 6 and get 2°. Subtract this from 63°,

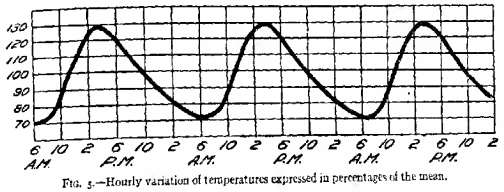

FIG. 5.—Hourly variation of temperatures expressed in percentages of the mean.

obtaining 61° as the probable mean temperature for the day. Turn now to Table II, where it is seen that the 3 p. m. temperature is 128 per cent of the mean. Hence multiply this percentage by 61° and get 78° as the probable temperature. This value could be improved much by getting the weather forecast from the Weather Bureau as explained earlier.

The first term of this equation is not 100 per cent, because the mean temperature of the day is not quite the average of the maximum and the minimum for the day.

To determine the temperature at 2 a. m., expressed as the percentage of the mean, divide 2 by 24 and multiply by 360°; 30° is obtained. Introduce 30 for θ in the above equation, and with the aid of a trigonometric table and a little multiplication the desired percentage will be obtained.

This equation will apply for any location where the mean daily temperature variation decreases as the mean daily temperature decreases, so as to give the ratio of the difference between the maximum and the mean to the mean a constant value of 1.29, as in Utah. The writers think it is of rather wide application.

The fact that this ratio comes out constant for all seasons is a coincidence, inasmuch as it holds only for the Fahrenheit scale of degrees and this scale was arbitrarily chosen.

The complete solution of the problem of determining the probable temperature at some particular place, such as Denver on June 15 at 2 p. m., would be to determine first the mean daily temperature for June 15 by means of equation 4, using as the first term of the equation the mean annual temperature for Denver and changing the date to degrees as explained earlier. By means of equation 6 just as it stands, change 2 p. m. to degrees and insert this value for θ in the equation and thus determine what percentage of the mean temperature the 2 p. m. temperature is. Multiply this percentage by the mean daily temperature obtained from the solution of equation 4 and get the desired 2 p. m. temperature.

The lowest monthly temperature given in Table I is for January at Uinta and is 16° F. With a daily variation of only 15°, a minimum of 8° would be expected. On certain days, however, negative temperatures are experienced. If the mean gets too low, some correction must be made, because the numerical solution does not, as it stands, allow negative values. When the mean temperature gets as low as 25° or lower add 20° to the mean, multiply by the percentage given in the table, and then substract the 20°; the normal temperature will be obtained. This will accommodate itself to negative values also.

The methods described above (particularly the trignonometric one) for determining the normal temperature give very accurate results for dry and humid regions. There are, however, two sources of error. The Weather Bureau record might not cover a long enough period of time, and the addition of another year's values might change the normal that we have used in making the equation. Also the equation, which is a series, might not include enough terms to have it represent exactly the normals. As explained earlier, these errors are very small.

Actual temperatures depart more from the normal in humid regions than in dry sections, and in comparing the normal as calculated by the foregoing methods with a particular observed temperature, the departure will be considerable in the humid section but only slight in dry regions. In the western part of the United States between the Rocky and the Sierra Nevada Mountains, an average yearly temperature departure exceeding 0.5° F. is unusual. In the average monthly temperatures, a departure from normal of 2° is common, but a departure of 4° is unusual. For the daily temperature, a departure of 4° from the normal is common, and a departure of 10° is unusual.

The States of Idaho, Nevada, Utah, Arizona, New Mexico, Montana, and Wyoming all have a relative humidity of about 50 per cent and an annual precipitation of from 10 to 20 inches—that is, the precipitation is light and the atmosphere comparatively clear. About 300 days out of 365, or about 80 per cent, are days free from rain, with the air clear and the sunshine bright most of the time. Better results, therefore, can be obtained in forecasting the temperature in this section of the country than in the sections where rains are frequent and the thermograph records are irregular in shape (see fig. 6).

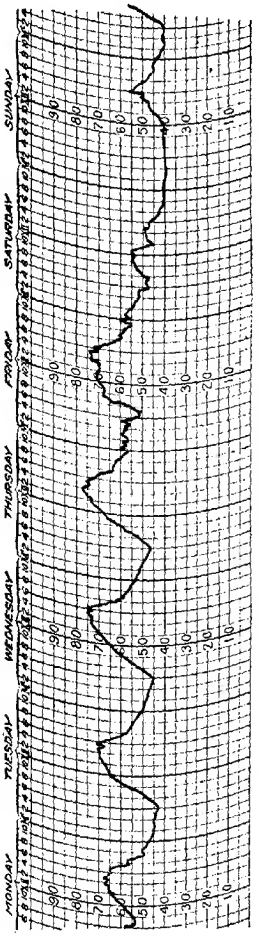

FIG. 6.—Effect of storm on diurnal variation of temperature.

Applying this rule and then checking the temperatures (not the normals) as they really occur, the writers found that the actual values differed from the theoretical value by from 1° to 15° F. with a mean departure of 7°. A very few errors were more than that. The chances in the arid West are 1 in 6 that the error will be less than 2°, 2 in 5 that the error will be 5°, 1 in 4 that it will be 10°, and 1 in 7 that it will be 15°.

SUMMARY

This paper gives an approximate solution of the problem of the determination of the normal temperature at a certain place at an assigned hour of the day on a particular day of the year.

An equation that shows the seasonal change in temperature is presented and gives the mean daily temperature in terms of the time of year. Another equation gives the percentage of the mean temperature that the temperature of a particular hour of the day is in terms of the time of day. The product of the results of the solution of the equations gives the temperature sought. The equation is of general application inasmuch as the first term is the mean annual temperature and the value for the location considered is to be inserted in the equation before using it.

An arithmetical solution is also presented. The hourly temperatures, expressed as percentages of the mean daily temperatures, are given. The mean monthly temperature for each of the 12 months must be known for the location considered. The mean daily temperature changes approximately one-third of 1° F. a day, except from December 15 to January 15 and from July 15 to August 15, when the mean temperature for any day is approximately equal to the mean temperature for the month With this information, the mean daily temperature is readily calculated, and by multiplying this value by the percentage found in the table for the particular hour considered, the desired probable temperature is obtained.

In the arid West the mean error of the method of determining the normal temperature is very small, but the mean error in predicting the actual temperature is 7° F., with 60 per cent of the errors less than this amount. These errors are due largely to the abnormal temperatures produced by rain and snowstorms.

TEMPERATURE RELATIONS OF CERTAIN POTATO-ROT AND WILT-PRODUCING FUNGI

By H. A. EDSON, *Pathologist*, and M. SHAPOVALOV, *Assistant Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Plant pathologists are well aware of the fact that certain parasitic organisms which seriously injure growing crops in one geographical latitude remain practically harmless in another. It seems reasonable to assume that the temperature of the different regions, aside from other climatic conditions, may greatly influence the occurrence of these parasites. As shown by Fawcett in his recent work,[1] a correlation exists between the cardinal temperatures of certain fungi in cultures and their geographical distribution and seasonal occurrence.

Moreover, the importance of properly regulated low temperatures for cold storage is fully recognized, not only for the purpose of controlling physiological and chemical changes, but also in order to check the progress of parasitic diseases. On the other hand, certain high temperatures are successfully employed not only to regulate ripening and sweating processes but also to effect the death of the invading parasites without lowering the vitality of the host.

There is, however, a regrettable lack of exact information regarding the temperature relations of different potato parasites. The following data secured in experiments with pure cultures of some of the most common potato-rot and wilt fungi may prove to be interesting and significant. They explain to a certain degree the predominance of these organisms in definite regions and definite seasons. They also permit certain practical conclusions regarding the temperatures which may control or eliminate these fungi.

CULTURES AND METHODS EMPLOYED

The cultures used in the tests with statements concerning their origin and time of isolation are listed below. As will be seen later from the graphs, the two strains of Verticillium, one from the South and the other from the North, showed distinctly different thermal behavior. Only one strain of each species of Fusarium was used in all experiments.

Fusarium coeruleum (Lib.) Sacc. (Culture No. 201).[2] Isolated by C. W. Carpenter in March, 1915, from potato tubers grown in the State of New York.

[1] FAWCETT, H. S., PRELIMINARY NOTE ON THE RELATION OF TEMPERATURE TO THE GROWTH OF CERTAIN PARASITIC FUNGI IN CULTURES. *In* Johns Hopkins Univ. Circ. 293 (n. s. no. 3) p. 191–194. 1917.

[2] Numbers accompanying each culture refer to the writers' catalogue.

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F. discolor, var. *sulphureum* (Schlecht) App. and Wollenw. (Culture No. 203). Dr. Wollenweber's culture isolated at Dahlem, Berlin, in June, 1909.

F. eumartii Carp. (Culture No. 204). Isolated by C. W. Carpenter in January, 1914, from a stem-end dry-rotting tuber grown in Pennsylvania.

F. oxysporum Schlecht. (Culture No. 208). Isolated by H. A. Edson in October, 1916, from potato tubers grown in New Jersey.

F. radicicola Wollenw. (Culture No. 211). Isolated by H. G. MacMillan in October, 1916, from potato tubers grown in Colorado.

F. trichothecioides Wollenw. (Culture No. 214). Isolated by O. A. Pratt in October 1916, from potato tubers grown in Idaho.

Verticillium albo-atrum Reinke and Berthold. (Culture No. 426). Isolated by C. W. Carpenter in September, 1915, from an eggplant grown at Shepherdstown, W. Va.

V. albo-atrum (Culture No. 427). Isolated by M. Shapovalov in September, 1917, from the vascular system of a wilted potato stem grown at Presque Isle, Me.

The growth of these fungi at various temperatures was studied in plates containing 10 cc. of a 2 per cent potato agar without sugar. A small drop of a water spore suspension of the respective fungus was placed in the center of each plate by means of a 2-mm. loop. The plates were then distributed in the incubators running at from 1° to 40° C., with approximately 5° difference between chambers. Additional sets of plates of *Fusarium oxysporum* and *F. radicicola* were kept at 37° and 39°, respectively, the maximum temperature here being of particular interest on account of a thermophylic habit of these organisms. Observations were made and measurements of the diameters of the colonies were taken daily for a period of two weeks. At the end of the first week the most rapidly growing colonies reached the edges of the plates; therefore further data were of little comparative value and were omitted in the final compilation. The tests were repeated three times in their entirety and in some inconclusive cases four and five times. The average results then were plotted and are presented in the accompanying graphs.

DISCUSSION OF RESULTS

A general examination of the figures 1 to 8, which represent daily accumulations of growth, at once reveals a peculiar common feature in the structure of the graphs: each series of curves characterizing the growth of a given organism originates at scattered points to the left, the lowest thermal points, then rises in the direction of the optimum temperature, and finally falls to a single point at the right, the highest thermal limit. *Fusarium oxysporum* and *Verticillium albo-atrum* No. 426 appear to be slight exceptions to this rule, but only for the first day. Quite a contrasting picture is seen in figure 9, where each curve shows the amount of growth produced by a corresponding organism for the total period of 7 days. Here, with but one exception, the entire series of curves has its origin at a single point to the left and is distributed at different points to the right. Thus, it is evident that the highest thermal point of growth was reached within the first 24 to 48 hours, while the lowest limit did not become apparent until the expiration of from 5 to 7 days.

The minimum temperature of growth and germination for the five Fusaria and the two Verticillia lies either somewhat above or somewhat below 5° C. *Fusarium discolor* var. *sulphureum* formed a small amount of visible growth at 5° on the sixth day, but neither growth nor germination could be seen at 1° after 7 days, although it was observed after 2 weeks. *F. oxysporum* did not grow and did not germinate at 5° even in two weeks. The remaining fungi did not produce visible growth at 5° in the first 7 days, but the germination was found to have taken place

FIG. 1.—Graph showing the rate of growth of *Fusarium coeruleum* on potato agar at different temperatures. The growth made during the first 24 hours is marked here with a dotted line. It was very indistinct because the fungus was not in high culture.

when plates were examined under the microscope. The length of the germ tubes, however, varied considerably with different organisms. This difference was particularly significant in the two Verticillia; the spores of the culture No. 426 produced only very short germ tubes, while the spores of the culture No. 427 formed a fair weave of fungal threads. Brooks and Cooley [1] report no germination with *F. radicicola* at 5° even after the expiration of 10 days, but they made their studies with corn-meal agar cultures and the results thus obtained may not be fully comparable with those here reported.

[1] BROOKS, Charles, and COOLEY, J. S. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. *In* Jour. Agr. Research, v. 8, no. 4, p. 139–164, 25 fig., 3 pl. 1917.

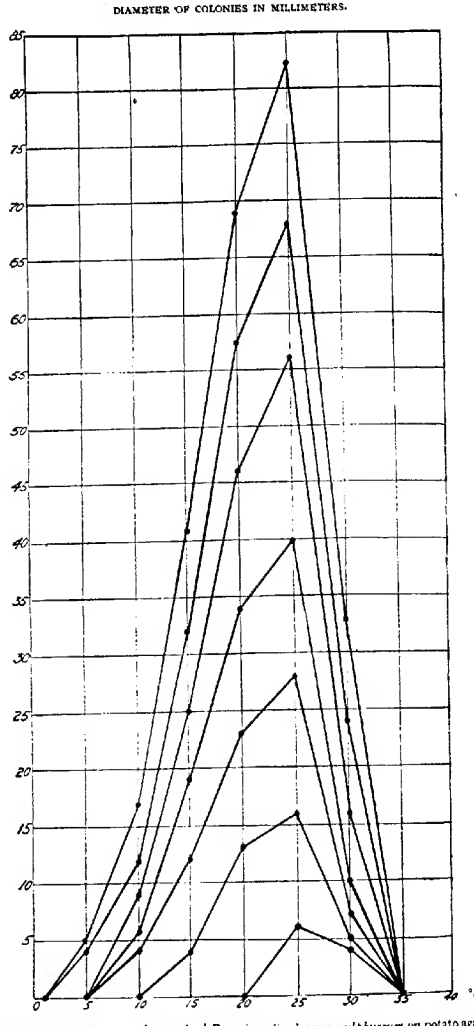

FIG. 2.—Graph showing the rate of growth of *Fusarium discolor* var. *sulphureum* on potato agar at different temperatures.

The optimum temperature divides the potato fungi into two groups. One, consisting of four Fusaria and two Verticillia, has its optimum in the neighborhood of 25° C.; and the other, comprised of *F. oxysporum* and *F. radicicola*, has its optimum in the vicinity of 30°. As shown by Brooks and Cooley,[1] the optimum temperature for *F. radicicola* on corn-meal agar is the same as stated here for cultures on potato agar—that is, 30°. After 7 days of incubation at this temperature the two latter parasites practically covered the plates. *F. discolor* var. *sulphureum* and *F. trichothecioides* produced the next largest amounts of growth, and the

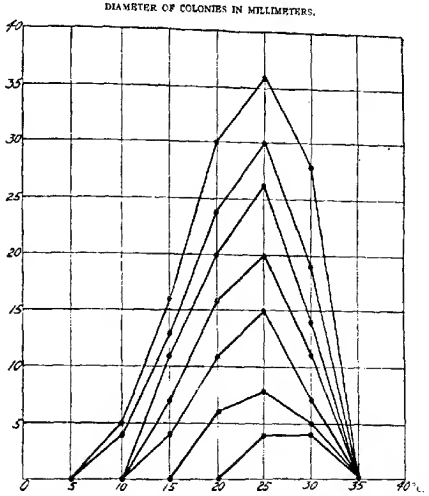

FIG. 1.—Graph showing the rate of growth of *Fusarium cumartii* on potato agar at different temperatures

remainder formed colonies measuring on the average only 22 to 32 mm. in diameter.

The maximum temperature at which growth occurred in *Fusarium coeruleum*, *F. trichothecioides*, and *Verticillium albo-atrum* No. 427 was at or slightly below 30° C. Germination took place with *F. trichothecioides* at 30°, but no growth visible to the unaided eye was formed. The other two fungi did not even germinate at this temperature. *F. discolor* var. *sulphureum*, *F. cumartii*, and *V. albo-atrum* No. 426 were not able to germinate at 35°. The Verticillium spores remained unchanged, but the normal typical spores of the two Fusaria were gradually changed to chlamydospores. Similar transformation of the spores of *F. oxysporum* occurred at 37° and of *F. radicicola* at 39°.

[1] BROOKS, Charles, and COOLEY, J. S. Op. cit., p. 156.

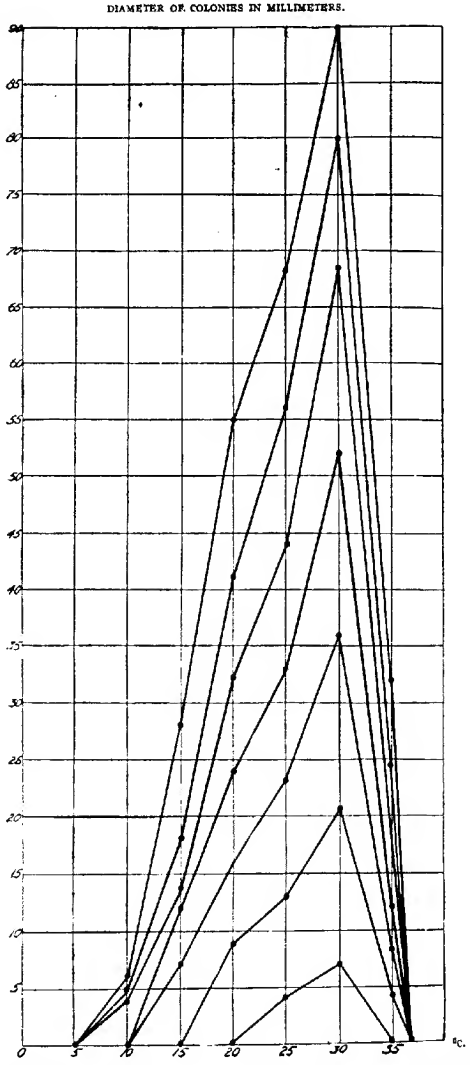

FIG. 4.—Graph showing the rate of growth of *Fusarium oxysporum* on potato agar at different temperatures.

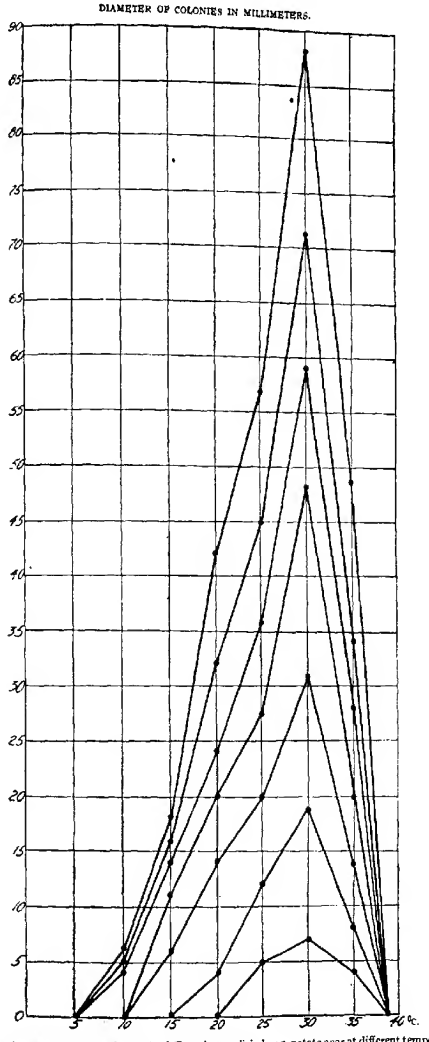

FIG. 5.—Graph showing the rate of growth of *Fusarium radicicola* on potato agar at different temperatures.

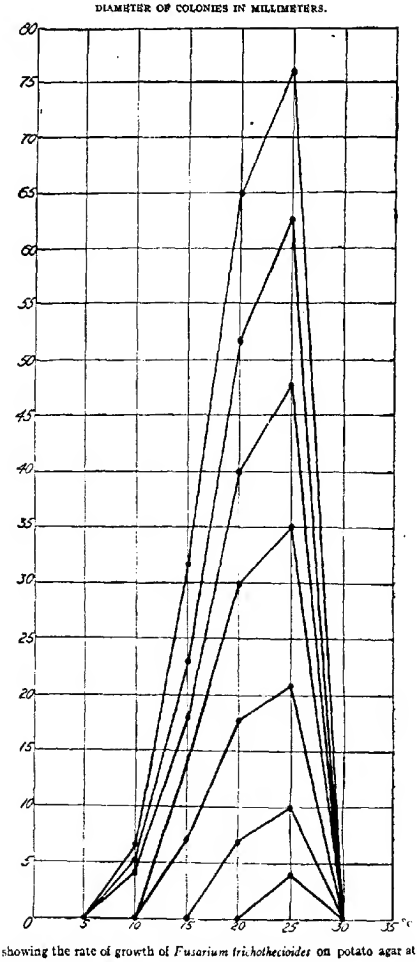

FIG. 6.—Graph showing the rate of growth of *Fusarium trichothecioides* on potato agar at different temperatures.

The initial growth after the first 24 hours was noted always either at the optimum point alone or at the optimum point and at one or two lower

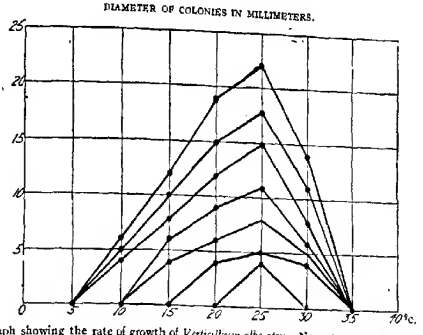

FIG. 7.—Graph showing the rate of growth of *Verticillium albo-atrum* No. 426 on potato agar at different temperatures.

or higher grades of temperature, but never exclusively at any point other that the optimum temperature (fig. 1–8).

Comparing the foregoing results of the temperature studies with the geographical distribution of the fungi, we find that the parasites prevailing

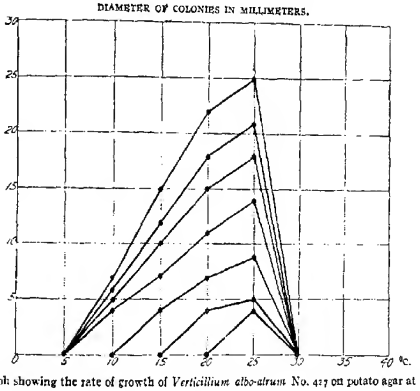

FIG. 8.—Graph showing the rate of growth of *Verticillium albo-atrum* No. 427 on potato agar at different temperatures.

in northern sections of the country (*Fusarium coeruleum* and *Verticillium albo-atrum* No. 427) have comparatively low maximum and low optimum points; those from central zones (*F. cumartii* and *V. albo-atrum* No. 426) exhibit a higher maximum, although they still retain practically the same

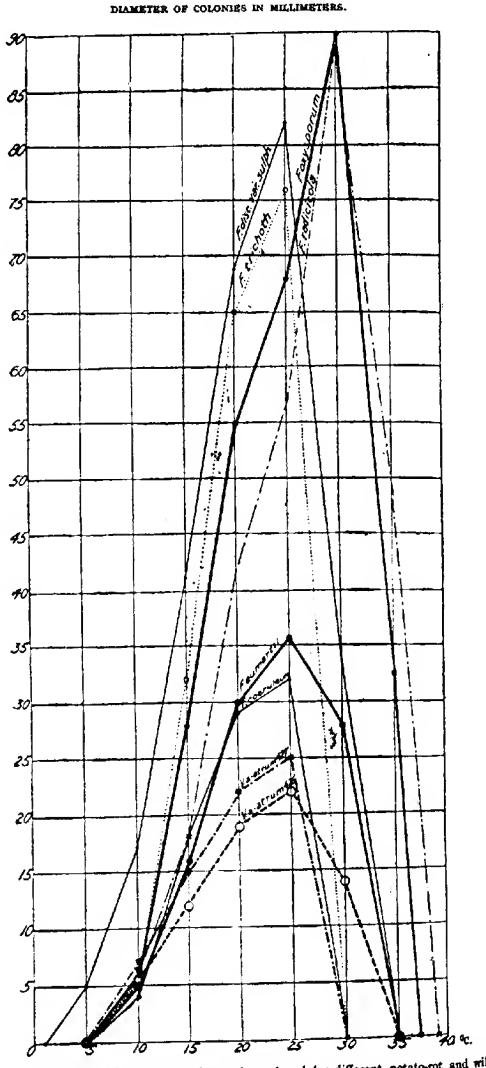

FIG. 9.—Graph showing the total amounts of growth produced by different potato-rot and wilt fungi during the first seven days on potato agar at different temperatures.

moderate optimum; while the parasites of the southern zones (*F. radicicola* and *F. oxysporum*) shift very decidedly toward both a higher optimum and a higher maximum temperature.[1] *F. discolor* var. *sulphureum* occupies a somewhat intermediate position between these groups, which may well explain its ubiquitous nature as a storage-rot organism. It has a comparatively wide thermal range of growth with a moderate optimum temperature. *F. trichothecioides* should be referred to the northern group of the fungi, taking as a basis its thermal behavior in artificial cultures. It differs, however, from the other two fungi of this group by its more rapid growth at and somewhat below the optimum temperature. This corresponds to the very active destruction caused by this fungus after it becomes well established in the host tissues. The two Verticillia give a particularly significant example of the correlation between the thermal response of the fungi and their geographical occurrence. The Northern strain shows a better adaptation to lower temperatures and does not grow above 30° C., while the southern strain shows a better adaptation to higher temperatures and gives a fair growth at 30°. Their morphological differences have not been sufficiently studied to warrant their separation as two different species. It may be stated, however, that they are not fully identical in pure cultures. Culture No. 426 has a tendency to produce numerous small sclerotia, which are practically absent in culture No. 427. The difference in response between the two wilt-producing fungi, *F. oxysporum* and *V. albo-atrum* No. 427, both with regard to their lower and higher growth limits and their optimum points, was even greater than with the two Verticillia. The results correlate with their geographical distribution and explain why *F. oxysporum*, although present in the soils of Maine, as well as on decaying tubers and in stem lesions of the potato there, has, nevertheless, not been associated with potato-wilt in that region.

A correlation can be drawn also between the temperature relations and the seasonal occurrence of *Fusarium oxysporum* and *Verticillium albo-atrum* No. 427, as shown by the following experiments conducted at Arlington Farm, Va. A certain number of tubers of the Irish Cobbler variety were divided into three groups. One group was inoculated with *F. oxysporum*, one with *V. albo-atrum*, and one was left uninoculated for control. They were planted on the farm plots on April 27. A second planting of similar groups was made on July 17. The first crop was dug on August 26 and the second crop on October 21. Immediately after digging the tubers were examined and wherever the stem-end discoloration was present, isolations were made. The results of these isolations are given in Table I.

[1] The zones referred to should not be regarded as definitely limited geographical areas. The terms employed are relative only, and the areas overlap. They are determined not only by latitude but by elevation and by soil climate. The fungi assigned to one zone may and usually do occur in the other. The assignment has been determined largely by the relative prevalence and parasitic virulence of the organisms.

TABLE I.—*Seasonal prevalence of Fusarium oxysporum and Verticillium albo-atrum under the climatic conditions of the vicinity of Washington, D. C.*

Treatment of seed tubers.	Early planting.						Late planting.					
	Total number of tubers.	Tubers showing discoloration.	Tubers giving cultures of—			Number sterile.	Total number of tubers.	Tubers showing discoloration.	Tubers giving cultures of—			Number sterile.
			F. oxysporum.	*V. albo-atrum.*	Miscellaneous fungi.				*F. oxysporum.*	*V. albo-atrum.*	Miscellaneous fungi.	
Inoculated with *F. oxysporum* a few days before planting	60	11	8	0	0	3	183	10	2	0	0	8
Inoculated with *V. albo-atrum* a few days before planting	53	30	3	1	8	18	159	44	3	7	2	29
Control tubers, uninoculated	46	18	8	0	0	10	110	16	1	0	0	15

It appears from this table that there were more tubers infected with *Fusarium oxysporum* in the early crop grown at higher temperature than in the late crop grown at lower temperature, and vice versa with *Verticillium albo-atrum*. The presence of the Fusarium infection in the control tubers indicates that, probably, the largest part of it came from the soil, while the infection of the new tubers with Verticillium came exclusively from the seed. Verticillium was absent both in the control plot and in the Fusarium plot of each crop.

The fact that the growth of potato fungi was seriously inhibited at or somewhat below 5° C. is of a considerable practical importance. Although the curves showing the temperature relations of these fungi in pure cultures may not always coincide with those which would designate their development in the host tissues, yet one may be reasonably certain that a temperature of about 40° F. or slightly below will suffice to check the spread of the Fusarium potato tuber-rots in storage. Brooks and Cooley's studies[1] show that the growth of apple-rot fungi is retarded by low temperatures to a much greater degree on the host itself than on artificial media The requisite temperature for successful infection is, therefore, higher than the minimum temperature necessary for growth in cultures.

High temperature treatment of the seed tubers to effect the death of invading parasites suggests itself as a thing which may deserve certain attention. In view of the thermophylic habit of *Fusarium oxysporum*, the possibility of application of sufficiently high temperature to cause the death of the parasite and not to injure the vitality of the tuber is quite remote. The case with *Verticillium albo-atrum* is, however, more hopeful. Certain preliminary experiments conducted by the writers

[1] BROOKS, Charles, and COOLEY, J. S. OP. CIT.

indicate that while if sufficient moisture is provided the spores and the mycelium of this organism remain alive at 30° C., they do not survive exposure for several days to a temperature of 35° or higher.

Several potato agar plates inoculated in the manner described above were kept at 30° and 35° C. for 7, 8, 11, and 14 days and then removed to room temperature. No growth ensued in any of these plates.

Nine beef-broth tubes were inoculated heavily with the spores of Verticillium and placed in incubators set at 25°, 30°, and 35° C. At the end of two days spores germinated at the two lower temperatures and formed growth visible to the unaided eye. Spores at 35° remained unchanged; but when one tube was brought to the laboratory, germination took place and growth developed. Two additional tube cultures were kept at the original temperature two weeks and then removed to the laboratory. No growth resulted in these cultures.

Thirty-five tubers selected in the field from hills badly affected with Verticillium-wilt in northern Maine were incubated at 25°, 30°, 35°, 37°, and 41° C., with 7 tubers to each compartment. After 13 days 2 tubers were taken from each lot, and cultures were made from the discolored stem ends. Twelve plantings from the material kept at the two lower temperatures yielded 5 cultures of *V. albo-atrum* while 7 remained sterile. No culture was obtained from material exposed to 35° or higher. Two of the remaining tubers of each lot were incubated 22 days longer, or for a total period of 35 days. Isolations from the 25° and 30° lots gave 6 cultures of Verticillium out of 12 plantings. The lots at 35° and 37° yielded sterile cultures. The tubers held at 41° were dead and blackened and were discarded. The remaining 3 tubers of each lot were kept for an additional 11 days. Isolations were made from the material held at 30°, 35°, and 37°. Three out of 9 plantings from the 30° material gave cultures of *V. albo-atrum*, while plantings from the tubers stored at higher temperatures proved sterile. Thus, the results were uniformly identical; the fungus survived exposure to 30°, but in no single case survived the exposure to 35°.

Incubation of freshly prepared culture plates at a properly chosen high temperature may serve as a useful practical method for the differentiation of the fungi which possess strikingly different maximum temperatures but which are otherwise very similar in cultural characteristics, particularly if they do not readily yield high cultures. Such is the case with *Fusarium coeruleum* and *F. radicicola*. They resemble each other very closely in color reactions on standard culture media, yet it usually requires a considerable length of time before typical spores of the former can be secured. If plate cultures placed at 35° C. for two or three days show no growth whatever, there is perfectly safe ground to assume that the fungus is not *F. radicicola*. This test may be very helpful when a prompt identification is desired or when additional evidence to support a conclusion is being sought.

CONCLUSIONS

(1) A certain degree of correlation exists between the temperature relations of some potato fungi in pure cultures and their geographical distribution and seasonal occurrence. The correlation is particularly striking in the wilt-producing fungi, *Fusarium oxysporum* and *Verticillium albo-atrum*.

(2) A temperature of about 40° F. should hold Fusarium tuber rots in check during storage.

(3) The susceptibility of *Verticillium albo-atrum* to high temperatures suggests the possibility of a heat treatment for infected seed tubers.

(4) Temperature tests in certain cases may serve as a useful supplementary method for the identification of fungi exhibiting contrasting thermal relationships.

GERMINATION OF BARLEY POLLEN

By STEPHEN ANTHONY, *formerly Assistant*, and HARRY V. HARLAN, *Agronomist in Barley Investigations, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The artificial germination of plant pollens has met with varying success. As a whole, the pollen of the Gramineae has proved very difficult to germinate. While experiments with the pollen of many plants have resulted not only in ready germination but in satisfactory methods of preserving the pollen for considerable periods of time, the results with many of the Gramineae have been far from satisfactory. This is especially true of self-fertilized forms, for many of which no artificial germination of pollen has been secured. The pollen of corn, on the other hand, was germinated readily by Andronescu (*1*).[1] It is believed that the pollen studies reported herein record the first artificial germination of barley pollen.

These studies grew out of a series of observations made by the junior author on the lateral florets of 2-row barley. The stamens of these florets are sometimes abortive, sometimes rudimentary, and sometimes well developed, producing abundant pollen. In an attempt to classify these variations, the question naturally arose as to whether or not this pollen was viable in all cases, and for such determination a reliable method of artificial germination obviously was desirable. It was to fill this need that the investigation was undertaken. The senior author was associated in barley investigations at that time, and the original project was intended to be a joint study. As it turned out, however, practically all of the laboratory studies were made by the senior author, while the field experiments were contributed by the junior author.

REVIEW OF THE LITERATURE

The volume of literature on the germination of pollen is so extensive that mention of only those papers having a special significance in their relation to the present studies will be included. These, for the most part, are cited at specific points in the text. According to older views, pollen fell into three classes: (1) pollen for which water alone was necessary for germination, (2) pollen which required, besides water, a chemical stimulant, (3) pollen which germinated in a solution of sugar of various concentrations. Mohl (*3*) in 1834 germinated pollen in water. Van

[1] Reference is made by number (italic) to "Literature cited," p. 535-536.

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Tieghem in 1869 (*11*) used an artificial medium. Jost (*2*), studying the physiology of pollen, concluded, from the fact that it germinates on the stigmas of other plants, that physical factors and not specific substances of the stigma were responsible for germination.

Jost (*3*), working with the pollen of Glyceria, Dactylis, and Secale, accidentally noticed in one of his experiments that pollen of these plants would germinate in close proximity to a drop of water. He was able thus to germinate the pollen of rye (*Secale cereale*) without any medium whatsoever.

Mangin (*7*) concluded that pollen of certain plants could germinate and grow from the food reserves within the pollen grain, while pollen of other plants did not grow well except in the presence of external nutrients.

EXPERIMENTS WITH SOLUTIONS

In the experiments of the authors the first trials of germination were made in water and solutions of sugar, agar, and other nutritive substances of various osmotic concentrations. It is unnecessary to report more of the aqueous tests than the behavior in sugar solutions. High concentrations resulted in plasmolysis. Low concentrations resulted in bursting in mature pollen. Immature pollen grains increased in size but did not burst. Mature pollen did not increase in size before bursting. In concentrations slightly less dense than those at which plasmolysis begins, small knobs were formed by the protrusion of the cell contents from the pore through which germination takes place. These knobs reached a length of from 2 to 4 μ but did not grow further. The knobs frequently were distended by continued absorption of water. If the water absorption is rapid, the bulging intine, which surrounds the extended cell contents, is ruptured before the knobs attain much size. Various stages of knob formation are shown in Plate 60.

EXPERIMENTS WITH MOIST CHAMBERS

When it became evident that germination was not likely to be secured in solutions, trials were made on various membranes of plant and animal origin. These experiments met with no success. Trials were made in moist chambers with and without membranes. The moisture in these chambers was supplied sometimes by drops of free water and sometimes by fragments of living plant tissue, the necessary humidity in the latter case arising from the evaporation from ruptured cells. No germination was obtained. If the cells became too moist, the pollen burst; if they became too dry, the pollen shrank.

The water adjustment of the pollen was so delicate that it seemed impossible to obtain a method of sufficient refinement to secure and maintain the proper conditions. If was found that the pollen on a slide could be readily killed, drowned as it were, by blowing one's breath upon it. The final success came from an observation the senior author made at Aber-

deen, Idaho, on opening flowers early in the morning, about the time when fertilization is most frequent. The stigmas, when viewed with a lens were seen to be covered with minute droplets of water, apparently condensed there by evaporation from other tissues more exposed to external heat. This observation led to the belief that germination was dependent purely upon physical factors and that the control of moisture was essential to artificial germination.

The conditions observed in the field were duplicated as nearly as possible in the laboratory. Pollen was taken from an anther ready to burst and dusted on a slide inside a loosely placed Van Tieghem cell. A piece of mesophyll from the leaf of the garden pea was placed in the cell to supply water. The cell was covered with a cover glass and the slide placed outside on the window ledge. The idea in this procedure was that as the condensation of the moisture progressed there would be a slow transition from a very low humidity to a very high one and eventually to the deposit of free water on the pollen. At some point between these extremes, conditions favorable to the growth of pollen would be met. Germination was secured inside of five minutes on the first attempt.

Later trials frequently resulted in large percentages of germination. Germination was accomplished both with mesophyll from green leaves and with free drops of water as sources of humidity.

Uncovering the moist chamber invariably led to failure; the knobs which had formed never attained any size, and the pollen subsequently died. When the pollen was left undisturbed for five minutes, examination showed germination amounting to 40 per cent. The tubes attained a length of from 60 to 100μ. No bursting of any tubes was noticed. The rest of the pollen was either starting to collapse or was in a normal state, apparently viable but not germinating. It was noticed that if the slide was resting on a cool medium—that is, a moistened filter paper—the pollen grains were swollen, and bursting was pronounced. The condensed droplets of moisture could be seen easily around the pollen grains, directly drowning them.

However, difficulties of germination did not disappear with a realization of the conditions necessary to secure growth, and the authors have not been able to bring all the factors under absolute control. The proper range of humidity must coincide with a certain range of temperature. The moisture supply must be ample to secure germination and yet the transition must be slow enough so that germination will be accomplished before the pollen is drowned. The delicacy of the water adjustment is difficult to realize. Plate 61, A, shows normal pollen grains and Plate 61, B, the same grains exposed for 2 minutes to the air. Some of the shrunken grains may still germinate; but with the loss of very little more water, germination becomes impossible. Perfectly normal pollen of tested germination was left uncovered on a dry slide for 10 minutes at a temperature of 20° C. The authors were unable to germinate

this pollen, either by artificial means or on the stigma. On the other hand, normal pollen grains will burst from too rapid and too extensive water absorption if the water condensation is too rapid. Weather conditions affect the growth of pollen both in the field and in the laboratory. Germination is accomplished with difficulty on cold, wet days. There is very little fertilization in the field under such conditions; and germination in the laboratory on such days is obtained with difficulty, because the necessary conditions are less easily secured and viable pollen is much harder to obtain.

FERTILIZATION IN THE FIELD

In order to correlate the laboratory experiments with fertilization under field conditions, extensive experiments were made by the junior author in 1917 and 1918. Both the period of receptivity of the stigma and the duration of viability of the pollen were studied. In the study of the receptivity of the stigma the results were strikingly uniform. Two hundred and eighty flowers were emasculated in one afternoon. For this purpose spikes were chosen which, by the length of the protruding awn and the first suggestion of the parting of the leaf sheath to release the side of the spike, indicated that fertilization would have occurred the following day. Forty flowers were pollinated immediately upon emasculation. Forty were pollinated on each succeeding day for six days. The results are shown in figure 1.

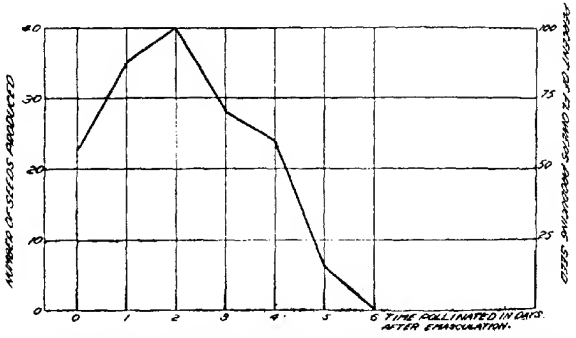

FIG. 1.—Period of receptivity of the stigma under field conditions as shown by the number of seed produced when 40 flowers were pollinated on emasculation and the same number on six successive days after emasculation.

The percentage of successful pollinations increased for two days. Of those pollinated two days after emasculation, 100 per cent of the ovaries set seed. From this time there was a gradual decrease, until on the sixth day no pollinations were successful. It is obvious that failures in

hybridization are due much more to faulty pollen than to any lack of receptivity of the stigma.

The viability of the pollen was tested both in 1917 and in 1918. As in the laboratory, the results were inconsistent, the variations indicating the readiness with which the viability of the pollen is affected. Forty flowers were used as a unit, as before, and all pollinations were made 48 hours after emasculation. In 1917, a different variety was selected for the test than was used in the experiment on receptivity of the stigma. Owing to the more advanced stage of the development of the plants, pollen was more difficult to obtain, and the highest percentage of successful pollinations was lower than in the previous experiment. The results are shown in figure 2.

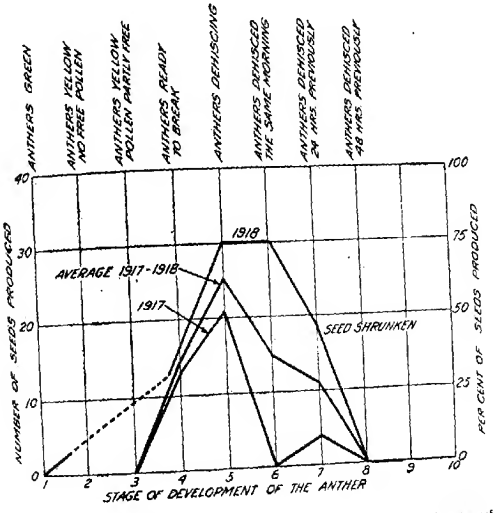

FIG. 2.—Number and percentage of seed produced by the use of pollen from anthers at various stages of development before and after dehiscence.

Anthers in eight different stages of development were used. In the first of these the anthers were green in color. In the second the anthers were yellow in color but did not yield dry pollen when broken. In the third the anthers were yellow in color, and pollen was secured from them only with difficulty. In the fourth stage the anthers were ready to break and yielded abundant dry pollen when ruptured. In the fifth stage the

anthers dehisced naturally. In the sixth stage the anthers had dehisced the same morning the filaments were extended. The base of the anther still contained considerable pollen, which was freed readily. The seventh stage was represented by anthers which had dehisced 24 hours previously. In this and in the eighth stage, in which the anthers had dehisced 48 hours previously, only a few still contained pollen. Only the best-appearing of this was used.

The period in which the pollen was found to be viable was very short. The best pollen was secured from anthers just breaking. Pollen from anthers ready to break gave a slightly lower percentage of successful pollinations. In 1918 the anthers used at this stage were slightly less mature than those used in 1917. Stamens with green anthers were used in both 1917 and 1918, but the two following stages were omitted in 1918. No seed was secured in either year from the use of immature pollen. These results are contrary to a widespread impression that detached anthers will complete the process of development, ripening, and fertilization when placed in a flower. By immature is not meant rudimentary pollen but pollen from anthers which, if left undisturbed, would not break for several hours. Pollen frequently can be dusted from such anthers by forcible rupturing of the walls. It will be recalled that this pollen swelled in water but did not burst. No artificial germination of such pollen was obtained.

The pollen remained highly viable in the field during only a few hours. Pollen taken from the anthers two or three hours before natural dehiscence effected fertilization in about 30 per cent of the attempts. Pollen taken from anthers which were dehiscing resulted in over 60 per cent of successful pollinations, while pollen remaining in anthers which had dehisced two or three hours previously effected fertilization in less than 40 per cent of the flowers pollinated.

In both years some fertilization resulted from pollen which had remained viable 24 hours after dehiscence. In one instance in 1918, not here reported, seed was secured from pollen of anthers which may have dehisced 48 hours previously. The age of the pollen in those classes after dehiscence is not certain. The stamens were left in the flowers, and the pollen remaining in the base of the anther was used. The age of the flowers was reckoned by means of observations on adjacent flowers and by other observations which have enabled the authors to determine the stage of development to within close limits.

In 1918 shrunken seed followed the use of pollen taken from anthers 24 hours after dehiscence. The numbers secured were not sufficient to indicate that the poor development was due to poor pollen.

It is the opinion of the authors that the success obtained with overripe pollen was due to the especially favorable conditions that prevail in Idaho. It is improbable, to the verge of certainty, that pollen could have remained viable in ruptured anthers 24 hours after dehiscence in a

humid climate. It would surely burst at night, when the condensation of moisture is heavy.

RETENTION OF VIABILITY IN THE LABORATORY

In the laboratory the attempts to keep barley pollen in such a way as to retain its viability met with no better success than in the field. Methods of keeping pollen of a few species of plants have been known for many years. Recently Pfundt (*9*) has been able to keep the pollen of many plants by regulating the amount of moisture in the air by means of solutions of dilute sulphuric acid of different water tensions. Swingle (*10*) and others have succeeded in keeping pollen by partial or complete drying in a vacuum. The laboratory attempts reported here were of three classes:

(1) Pollen left in the free air at 18° C. for different periods of time

(2) Pollen kept over sulphuric acid of different strengths.

(3) Pollen partially or completely dried in vacua at different temperatures.

In all cases the viability of the pollen was ascertained not only by artificial methods of germination but also by testing the germinative power directly upon the barley stigma. It was found advantageous to follow the growth by means of reagents, although the preliminary stages are characteristic and readily seen. Pollen brought in contact with the stigma attaches itself by means of some adhesive substance present, not only on the entire surface of the stigma hairs but also on the pollen grain itself. Normal viable pollen shows a distinct swelling, evidenced by different bulgings of the surface of the grain, which gradually disappear. The swelling is followed by a protrusion of the tube. This protrusion is very short, the tube bending immediately upon the grain, exhibiting a distinct contact tropism. The rapidly growing tube soon enters and disappears within the stigma hairs. The whole process of germination lasts from two to four minutes. To follow and differentiate the tube, the preparations were stained with a dilute solution of methyl-green acetic acid. This stains the pollen grain bluish and the contents of the tube light green. If a dilute solution of Congo red is applied subsequently, the tube is colored red, while the grain, which is unaffected, remains blue. Prolonged staining with Congo red will color the grain violet, the stain being so stable that it persists even after washing with a 5 per cent solution of sodium carbonate. Pollen in various stages of germination is shown in Plate 60. In Plate 60, the tube is still attached to the stigma, but the pollen grain has been loosened so as to make it more easily distinguished.

POLLEN LEFT IN FREE AIR

Pollen left exposed to the air for only two minutes loses so much moisture that it becomes shrunken. Shrunken pollen, however, does not mean necessarily a nonviable pollen. Such pollen may germinate,

provided its moisture content does not reach a dangerously low level. Normal pollen is shown in Plate 61, A. The same pollen exposed for 2 minutes to the air is shown in Plate 61, B. Some of this pollen may germinate. As previously stated, however, when the pollen was exposed to the air for 10 minutes, it had lost its germinative properties completely. This shrunken pollen, when applied to the stigma, affected the stigma hairs noticeably. The pollen grains took up moisture, attaining the round appearance of normal pollen; but the stigma hair cells lost their turgor and became vacuolized. It was thought that this effect upon the stigma hair cells might be overcome by swelling the pollen previous to its application to the stigma, but pollen so treated did not germinate.

Since the viability was lost so quickly in free air, it was obvious that more elaborate experiments of this nature were useless. While not strictly in free air, pollen remained viable 24 hours in a cool, dark room, when inclosed in a loose Van Tieghem cell with a piece of pea leaf as a moisture-giving medium.

POLLEN KEPT OVER SULPHURIC ACID

The trials in free air indicated that the regulation of the moisture content of the air was perhaps the most important factor in preserving pollen. Pollen was kept over sulphuric acid of different concentration as follows:

15.14 per cent with a 90 per cent moisture saturation;

37.69 per cent with a 60 per cent moisture saturation;

54.00 per cent with a 30 per cent moisture saturation.

Germination was later secured only from the 90 per cent moisture concentration. The experiment was not satisfactory in that the control was not accurate. This was especially true of the temperature. From all the observations made, it is the opinion of the authors that in the presence of a considerable range of humidity, pollen must remain viable for some time at a temperature of about 10° C.

POLLEN KEPT IN VACUA

The consequence of exposing pollen to the air were so obvious that experiments in vacua seemed foredoomed to failure; but citrus pollen had been preserved so successfully in this way that the same method was employed with barley pollen.

The pollen was put in gelatin capsules, care being taken not to have them completely closed. The capsules were placed in test tubes, which were subsequently evacuated, the vacuum being regulated to the millimeter pressure wanted. In no instance did barley pollen remain viable. The pollen was invariably shrunken and would not germinate under any conditions.

DISCUSSION OF RESULTS

The outstanding feature of all experiments with barley pollen is the extreme delicacy of the water adjustment of the pollen grain. Exposed to dry air for two or three minutes, the walls collapse through the loss of moisture. Exposed in a saturated atmosphere, the same cell imbibes water so fast that it bursts in even less time.

The water content of barley pollen is not known to the writers, nor has the percentage or the rate of loss of moisture been ascertained. It is known, however, that pollen of *Zea mays*, left under natural atmospheric conditions, loses from 40 to 50 per cent of moisture in a short time. Barley pollen may lose even higher percentages in the same length of time, if its sensibility to water is used as a criterion.

The imbibition of water occurs irrespective of the age of the pollen and probably occurs in nonviable as readily as in viable pollen. In aqueous solutions, immature pollen took up water and increased in size. Mature pollen under the same circumstances took up water but did not increase in size. The expansion of the contents resulted in bursting or in the protrusion through the pore of the intine and part of the cell contents. It seems logical to conclude that the cell wall of immature pollen becomes hardened by full maturity so that it resists the pressure of expansion.

When mature pollen is applied to the stigma the cell wall becomes cemented to the stigma hair, effecting a union sufficiently strong to furnish an anchorage from which forcible entry can be made into the stigma hairs.

The actual germination or growth is a complex process attributable to the specific physicochemical properties of the protoplasm and its properties of imbibition. The imbibition pressure is a deciding factor in primary growth. McDougal (6) believes this is the case in growing cells in other tissues. Osmotic pressure apparently is a negligible quantity at first, but it is of high importance in the later stages of growth.

The protoplasm is considered an emulsion colloid of a diphasic character and reversible in reaction. It possesses highly hydrophilic properties, and because of its high viscosity possesses also a high diffusibility for water. The behavior of barley pollen under the diverse physical conditions of moisture and temperature leads to the assumption that the reversible system from sol to gel is not pronounced, minute changes of moisture being able to destroy completely the balance of the system in one direction. It is true that germination may be accomplished after the cell walls have begun to collapse, if the loss of water has not reached the point where irreversible processes may take place. The loss of viability of barley pollen left for several minutes in free air may also be explained by the formation of synthetic products dehydrolitic in character, which are toxic and inhibit germination.

What part is played by the lipoid content of the protoplasm and its spatial relation to the disperse medium in those different colloidal processes we can only surmise, since the chemistry or even the quantitative analysis of pollen is not well known. Lloyd (*4, 5*) infers that there may be such a relation.

The extreme sensitiveness of the pollen grain of barley to external conditions might be expected to lead to considerable sterility in the field. In most varieties, however, there is very little sterility. This, doubtless, is due to an equally delicate adjustment of the dehiscence of the anther. Development of the anthers seems to be entirely checked on cold, wet days. There may be very little fertilization for two or three days in succession during a period of adverse weather conditions. The development or at least the dehiscence of the anthers is retarded at night as well. In the morning, especially if the morning be clear, there is a very rapid evaporation of the dew and other condensed moisture from the surface of the plant. A little later the anthers begin to dehisce. This rupturing of the anther apparently occurs when its temperature rises and its surface begins to dry. This seems to be the time most favorable for the growth of the pollen as well. Conditions unfavorable for the growth of pollen are unfavorable for dehiscence.

The large number of anthers breaking in the early part of the forenoon indicates that those ripening since the preceding day had delayed dehiscence until favorable conditions obtained. After the very active period of the forenoon little fertilization occurs until midafternoon. It would seem that most of the anthers near ripeness had burst during the favorable period of the morning; and, except in scattering florets, renewed activity had to await the maturation of the next group of anthers. It is also possible that the extreme temperatures of midday may retard dehiscence.

There are a few varieties which exhibit infertility in the field. Primus, perhaps, is the most conspicuous of these. In Minnesota, in some years, finely developed spikes may produce but three or four kernels. In the West this variety is much more fertile. The cause of its infertility in Minnesota has not been ascertained by the writers, since neither of them has been at the St. Paul Station at flowering time since the pollen experiments were developed. It may be that the anthers burst at inopportune times or that many stamens may remain undeveloped. The senior author found the latter condition to exist in the greenhouse in Washington.

The experiments in keeping barley pollen did not lend any encouragement in this phase of the study. In practice, two suggestions may be offered. Pollen can be kept several days by picking the spikes just before the pollen matures and putting them in an ice box. The low temperature will prevent further development for a time. When needed, they can be placed with the stems in a glass of water in a warm room,

and they then will complete their development. If it is apparent sometime in advance that the desired varieties are not going to flower at the same time, the spikes of the more advanced variety should be removed. This will cause a good development of the secondary culms, which may flower about the time of the later sort.

The methods developed for the germination of barley pollen appeared to offer advantages which might be used with other species of grasses. Large percentages of germination were obtained with wheat pollen under conditions identical with those used for germination of barley pollen, except that the pollen of wheat was slightly less sensitive to the moisture.

SUMMARY

The artificial germinination of pollen of grasses has long been known to be a difficult matter. In a study of the factors affecting viability of barley pollen, its relation to moisture was found to be very delicate. Slight drying caused collapse of the walls, and free moisture caused rapid swelling and bursting. The relation of temperature also is critical.

Experiments were conducted to determine viability in various solutions and in different moist chambers. The former resulted in failures; the latter, in success under delicately controlled conditions. A proper range of humidity must coincide with a certain range of temperature.

Extensive studies were made of fertilization under field conditions, using pollen in eight successive stages of development, from immature to that obtained two days after dehiscence. Sixty per cent of seed formation was obtained when ripe pollen was used.

The retention of viability by barley pollen when stored under various conditions was studied. Results were determined by artificial germination and by germination on the stigma. No satisfactory results were obtained.

Study of the conditions governing fertilization in nature shows that conditions unfavorable to fertilizations are also unfavorable to progress in the development of pollen and vice versa. In this way natural fertilization is assured.

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PLATE 60

A.—Normal pollen.

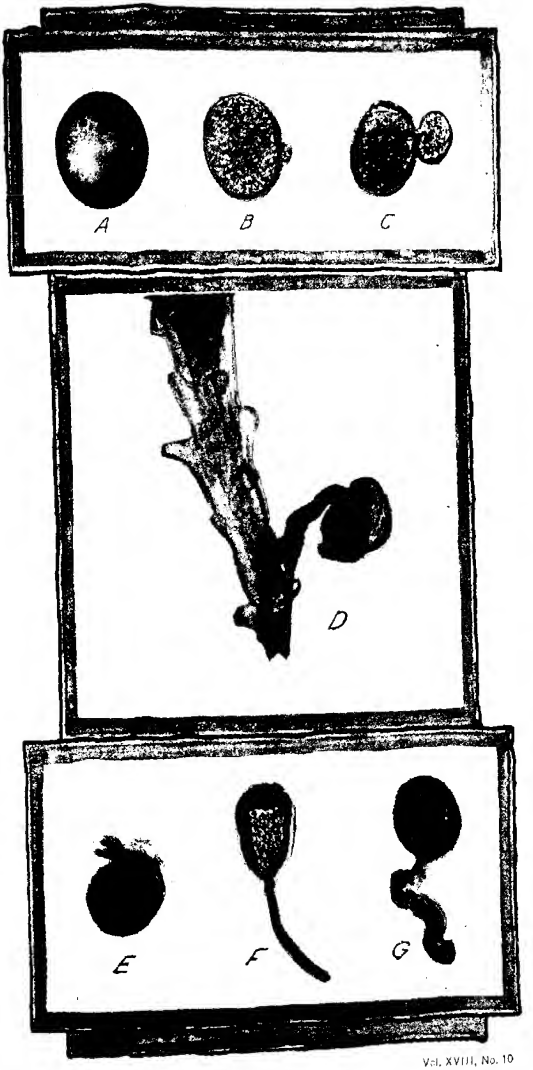
B.—Bulging of the intine through imbibition.

C.—The effect of free water when the intine is not ruptured.

D.—Pollen germinating on a stigma hair; the pollen grain loosened so as to show the tube.

E.—Germinating pollen.

F, G.—Pollen tube.


A
B
C
D
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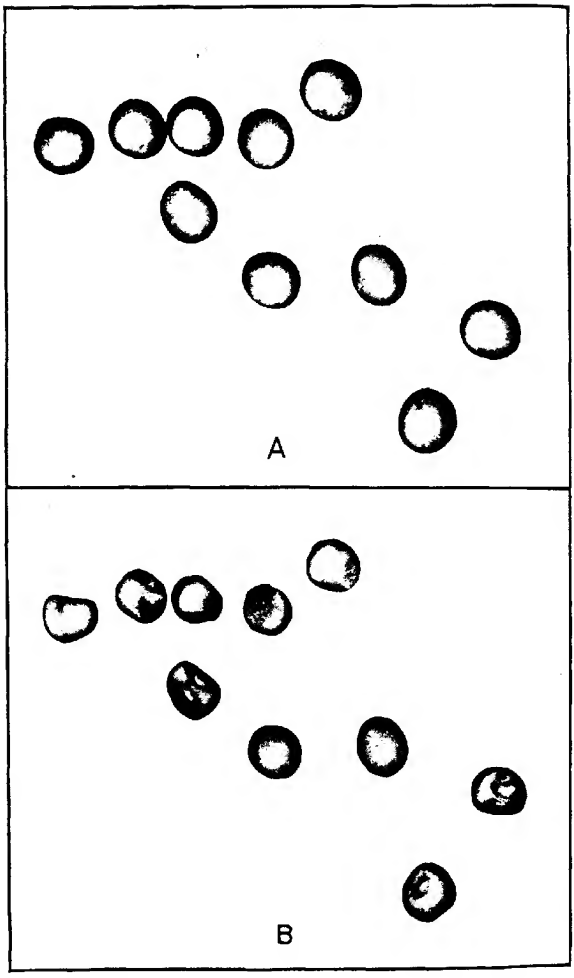
A
B

PLATE 61

A.—Normal pollen grains.
B.—Same pollen grains after an exposure of two minutes to dry air.

INVERTASE ACTIVITY OF MOLD SPORES AS AFFECTED BY CONCENTRATION AND AMOUNT OF INOCULUM

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It has previously been shown [2] that mold spores may release sufficient invertase to cause an appreciable inversion in 10 and 20 per cent sucrose solutions. This phenomenon was studied in connection with the deterioration of cane sugar by fungi [3], but in considering its bearing upon the latter problem it becomes imperative to establish the limits of concentration at which the invertase of mold spores is active. The work of O'Sullivan and Thompson [4] is responsible for the claim that invertase is not active in sucrose solutions above 40 per cent. The writers have been unable to find in the literature any contrary stand, although there exists some indirect evidence on this point. Since the film surrounding the sugar crystal capable of supporting biologic activity must vary in concentration from a supersaturated solution down to comparatively low concentrations (depending upon the amount and distribution of the moisture present), it was considered advisable to employ concentrations of 10, 20, 30, 40, 50, 60, and 70 per cent, at an incubation temperature of 48° to 50° C. for three days. The spore suspensions were prepared according to the method previously described.[2]

The experiment with blue aspergillus [5] is reported in Table I.

In Table I are presented the averages of closely agreeing triplicate determinations obtained with the spores of the blue aspergillus, at the rate of 144,000 spores per cubic centimeter for 10 to 40 per cent solutions of pure sucrose, and 130,000 spores per cubic centimeter for 50 to 70 per cent solutions. It will be noted that with an increase in concentration up to 60 per cent there is a proportional loss in percentage of sucrose and correponding increase in reducing sugars. Beyond this point the amount of inversion decreases. This brings out two important facts—namely, that the invertase in the spores of this mold is active in a sugar solution at the point of saturation, and furthermore that the maximum activity occurs between 50 and 60 per cent concentration for such an inoculation. It may be mentioned, parenthetically, that the polarization

[1] The authors are indebted to Lillian Kopeloff and the Station staff for many valuable suggestions.

[2] KOPELOFF, Nicholas, and KOPELOFF, Lillian. DO MOLD SPORES CONTAIN ENZYMS? *In* Jour. Agr. Research, v. 18, no. 4, p. 195–209. 1919.

[3] ——— ——— THE DETERIORATION OF CANE SUGAR BY FUNGI. La. Agr. Exp. Sta. Bul. 166, 72 p. 1919. Literature cited, p. 69–72.

[4] O'SULLIVAN, C., and THOMPSON, Frederick W. INVERTASE: A CONTRIBUTION TO THE HISTORY OF AN ENZYME OR UNORGANIZED FERMENT. *In* Jour. Chem. Soc. London, v. 57, p. 834–931. 4 pl. 1890.

[5] Now identified as *Aspergillus sydowi* Bain.

of 10 to 40 per cent solutions was made directly (using a 100-mm. observation tube where necessary) while 26 gm. in 100 cc. were polarized in the 50 to 70 per cent solutions. Consequently, the conversion of polarization to percentage of sucrose in the 40 per cent solutions is a calculation based upon an assumed Brix and specific gravity.

TABLE I.—*Influence of concentration on invertase activity of spores of blue aspergillus*[a]

Concentration.	Polarization.	Loss in polarization.	Loss in sucrose.	Reducing sugars.	Gain in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 per cent, control	44.2	.....	.....	0.03	.....
10 per cent, with heated spores	44.0	0.2	.....	.03	.....
10 per cent, with spores	34.1	5.9	1.5	1.79	1.76
20 per cent, control	85.1	.....	.....	.07	.....
20 per cent, with spores	71.9	13.2	3.2	2.78	2.71
30 per cent, control	124.6	.....	.....	.12	.....
30 per cent, with spores	107.3	17.3	4.0	2.78	2.66
40 per cent, control	166.6	.....	.....	.14	.....
40 per cent, with spores	147.7	18.9	4.2	2.94	2.80
50 per cent, control	52.8	.....	.....	1.00	.....
50 per cent, with spores	47.4	5.4	5.4	4.41	3.41
60 per cent, control	57.7	.....	.....	1.71	.....
60 per cent, with spores	54.6	3.1	3.1	4.17	2.46
70 per cent, control	66.1	.....	4.6	2.13	.....
70 per cent, with spores	61.5	4.6	.....	4.54	2.41

[a] 144,000 spores per cubic centimeter were added to the 10 to 40 per cent solutions and 130,000 spores per cubic centimeter to the 50 to 70 per cent solutions.

The results of the experiment with the spores of *Penicillium expansum* are given in Table II.

TABLE II.—*Influence of concentration on invertase activity of spores of Penicillium expansum*[a]

Concentration.	Polarization.	Loss in polarization.	Loss in sucrose.	Reducing sugars.	Gain in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 per cent, control	40.0	.....	.....	0.03	.....
10 per cent, with heated spores	40.0	.....	.....	.03	.....
10 per cent, with spores	24.8	15.2	3.8	2.77	2.74
20 per cent, control	82.5	.....	.....	.17	.....
20 per cent, with spores	58.3	24.2	5.8	4.17	4.00
30 per cent, control	122.6	.....	.....	.21	.....
30 per cent, with spores	95.3	27.3	6.4	5.00	4.79
40 per cent, control	170.7	.....	.....	.23	.....
40 per cent, with spores	128.2	42.5	9.5	5.77	5.24
50 per cent, control	53.0	.....	.....	.97	.....
50 per cent, with spores	49.9	3.1	3.1	2.87	1.90
60 per cent, control	57.7	.....	.....	1.71	.....
60 per cent, with spores	57.6	.1	.1	2.63	.92
70 per cent, control	66.1	.....	.....	2.13	.....
70 per cent, with spores	64.5	1.6	1.6	2.73	.60

[a] 5,600,000 spores per cubic centimeter were applied to the 10 to 40 per cent solutions and 220,000 spores per cubic centimeter to the 50 to 70 per cent solutions.

It is to be regretted that the inoculations differed so widely, for there were 5,600,000 spores per cubic centimeter in the 10 to 40 per cent solutions and only 220,000 spores per cubic centimeter in the 50 to 70 per cent solutions. However, it is evident that with an increase in concentration up to 50 per cent there is a marked loss in sucrose accompanied by a corresponding increase in reducing sugars. Beyond this point the loss in sucrose is not so significant. The same is true of the gain in reducing sugars. One may be permitted the speculation that had the inoculation in the 50 to 70 per cent solutions been 25 times larger, the results would have corroborated those previously obtained with the blue aspergillus—namely, that the invertase released by the spores of *Penicillium expansum* was active in concentrations of sugar as high as the saturation point and attained a maximum between the 50 and 60 per cent concentrations.

In Table III are recorded the results obtained with *Aspergillus niger* in concentrations above 50 per cent sucrose, since we have previously indicated an increase in invertase activity of the spores of this organism with an increase in concentration.[1]

TABLE III.—*Influence of concentration on the invertase activity of spores of Aspergillus niger* [a]

Concentration.	Polarization.	Loss in polarization.	Loss in sucrose.	Reducing sugars.	Gain in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
50 per cent, control	52.0	.....	.....	1.16	.....
50 per cent, with heated spores	52.0	.....	.....	1.40	0.24
50 per cent, with spores	40.6	11.4	11.4	6.94	5.54
60 per cent, control	57.7	.....	.....	1.71	.....
60 per cent, with spores	48.1	9.6	9.6	6.67	4.96
70 per cent, control	66.1	.....	.....	2.13	.....
70 per cent, with spores	57.0	7.1	7.1	5.18	3.05

[a] 680,000 spores per cubic centimeter were used.

It will be seen from Table III that the percentages of sucrose lost and of reducing sugars gained show a maximum at 50 per cent concentration with *Aspergillus niger*, and that there is a diminished but significant activity up to the saturation point as with the blue aspergillus and *Aspergillus niger*.

While it is indeed difficult to draw any comparative conclusions concerning the invertase activity of the three molds under consideration, because of the differences in the number of spores used for inoculation, nevertheless it can safely be postulated that the spores of the blue aspergillus are relatively more active in inverting power than the other two molds employed. For it will be seen that with 130,000 spores of this

[1] KOPELOFF, Nicholas, and KOPELOFF, Lillian. THE DETERIORATION OF CANE SUGAR BY FUNGI. La. Agr. Exp. Sta. Bul. 166, 72 p. 1919. Literature cited, p. 69–72.

mold per cubic centimeter at the highest concentration employed (which was at the saturation point) there was a gain of 2.41 per cent in reducing sugars over the control, while with double the number of spores of *Penicillium expansum* there was only one-fourth that gain in reducing sugars. Five times as many spores of *Aspergillus niger* produced one-fourth greater increase in reducing sugars.

In general, then, it will be seen that the invertase activity of these mold spores is directly influenced by concentration in the manner described, and furthermore that it is likewise dependent on the number of spores employed. The second part of this paper, therefore, is concerned with the influence of numbers of mold spores upon their invertase activity in sugar solutions at the saturation point.

Since there has been occasion in another connection [1] to study the influence of the number of mold spores, somewhat the same procedure was employed in this experiment. The spore suspensions were prepared as described in an earlier paper,[2] and the proper dilutions made with sterile distilled water. The same conditions of incubation, etc., were again observed. The control flasks contained a mixture of spores of all dilutions heated to 100° C. for ½ hour. The largest number of spores of *Penicillium expansum* and *Aspergillus niger* was about 400,000 per cubic centimeter, and the largest number of blue aspergillus was about 80,000. The dilutions represented an arithmetic progression of one-half of the preceding quantity.

In Table IV will be found results obtained with varying quantities of inoculum in a sugar solution at the point of saturation.

Some striking differences are to be noted where the blue aspergillus was used. With a decrease in quantity of inoculum there is a proportional decrease in invertase activity until 5,000 spores are employed, at which point no significant inversion occurs. Consequently that number may be taken as the lowest limit essential to invertase activity in such a highly concentrated sugar solution.

On the other hand, *Penicillium expansum* is not nearly so effective, for it will be seen that while there is a decrease in invertase activity with a decrease in number of spores, nevertheless the minimum is reached between 110,000 and 55,000 per cubic centimeter, which is practically 10 times as great an amount as was required by the blue aspergillus. The lower limits of the invertase activity of *Penicillium expansum*, however, are very sharply defined. The results obtained with *Aspergillus niger* are practically identical qualitatively with those observed for *Penicillium expansum*, but vary in the quantitative relationships. To a certain degree this is probably accounted for by the fact that the latter mold had the larger inoculation.

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[2] —— and KOPELOFF, Lillian. DO MOLD SPORES CONTAIN ENZYMS? *In* Jour. Agr. Research, v. 18, no. 2, p. 195–209. 1919.

TABLE IV.—*The influence of numbers of spores on the invertase activity of mold spores in 70 per cent sugar solutions*

BLUE ASPERGILLUS

Number of spores per cubic centimeter of solution.	Polarization.	Loss in sucrose.	Reducing sugars.	Gain in reducing sugars.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Control	66. 5	.....	0. 97	.....
80,000	59. 7	6. 8	4. 55	3. 85
40,000	63. 1	3. 4	2. 78	1. 81
20,000	64. 7	1. 8	2. 18	1. 21
10,000	65. 0	.5	1. 50	. 53
5,000	65. 3	1. 0	1. 07	. 10

PENICILLIUM EXPANSUM

Control	66. 3	.....	1. 50	.....
440,000	61. 1	5. 2	3. 57	2. 07
220,000	63. 8	2. 5	2. 67	1. 17
110,000	64. 9	1. 4	1. 90	0. 40
55,000	65. 2	1. 1	1. 48	0
28,000	66. 1	. 2	1. 48	0

ASPERGILLUS NIGER

Control	66. 4	.....	0. 81	.....
400,000	64. 0	2. 4	1. 31	0. 50
200,000	65. 3	1. 1	1. 00	. 19
100,000	66. 5	.....	1. 07	. 26
50,000	66. 3	. 1	. 86	. 05
25,000	66. 3	. 1	. 88	. 07

While the limitations of the data herein presented are clearly recognized to the extent that it is known to be exceedingly hazardous to state the limits of enzymic activity in terms of such units, because of the variations which must exist between different strains of the same species, nevertheless the results are suggestive in their bearing upon the practical problem of sugar deterioration. A certain correlation to be emphasized is that the blue aspergillus, which was found to have the greatest capacity for deteriorating sugar (besides occurring with greatest frequency), has spores which appear to exhibit the most intense invertase activity in saturated sugar solutions. Since we have pointed out that sugar inoculated with spores of this mold deteriorated without the development of mycelia, the conclusion would appear to be substantiated that mold spores alone, if present in sufficient number, are capable of deteriorating sugar.

SUMMARY

(1) The invertase activity of the spores of blue aspergillus, *Aspergillus niger*, and *Penicillium expansum* is exhibited at concentrations of sugar varying from 10 to 70 per cent.

(2) The maximum invertase activity of those mold spores occurs between 50 and 60 per cent concentrations.

(3) An increase in the number of mold spores is responsible for increased invertase activity in a saturated sugar solution.

(4) The least number of spores of *Penicillium expansum* and *Aspergillus niger* required per cubic centimeter to produce inversion in saturated sugar solution is between 50,000 and 110,000. About 5,000 spores of blue aspergillus are needed to cause inversion.

(5) The evidence that mold spores alone are capable of deteriorating cane sugar is corroborated by the data herein presented.

BASAL GLUMEROT OF WHEAT

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In the course of an examination in this laboratory of various collections of wheat of the crop of 1917 made for the study of "black chaff," a bacterial disease unlike "black chaff" or any other reported wheat disease was discovered; and this same disease was observed again several times in the collections of 1918.

This disease affects the leaf, head, and grain of wheat. On the heads the glumes show at the base a dull brownish black area. Sometimes this dark area extends over nearly the whole surface of the glume; but usually only the lower third, or less, is darkened (Pl. 62, A, B); and often no discoloration is visible on the exterior. Glumes that have a normal color on the outer surface may have the inner surface discolored. In practically all cases, dissection of the spikelet reveals more signs of disease on the inner surfaces than on the outer. Often a narrow dark line at the junction of the spikelet and the rachis is the only outward sign of the disease.

The grain inclosed by such diseased glumes shows varying degrees of undevelopment. The fact that grains are often well filled out would indicate that the disease sometimes appears late in the course of growth. In diseased grain the base, or germ end, varies in color from a scarcely noticeable brown to charcoal black (Pl. 62, C). In severe cases the surface texture as well as the color suggests charring. In the discolored areas bacteria are found in great abundance. Pure cultures have been secured from material collected in various States and in Canada.

This type of disease has been found in collections of wheat from New York, Michigan, Kansas, Missouri, Minnesota, North Dakota, South Dakota, Oklahoma, and Alberta, Canada.

The bacterium isolated from the infected glumes and grains is a medium-sized rod (1 to 2.7μ by 0.6μ), with rounded ends; in favorable media it forms long chains; it is actively motile by means of one to four polar or bipolar flagella (Pl. 63, F); and capsules are present in 6-day-old beef agar cultures (Ribbert's capsule stain).

No spores, zoogloeae, or involution forms have been observed.

The organism is Gram-negative and is not acid-fast.

Its staining reactions are rather feeble, but hot carbol fuchsin, anilin gentian violet, and saturated gentian violet gave good results. The stains must be washed out only with weak grades of alcohol (40 to 50 per cent), since strong alcohol takes them out too readily.

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Growth is good in the usual culture media, though the peptone-beef media are less favorable for growth and retention of vitality than potato, milk, or Uschinsky's solution.

CULTURAL CHARACTERS

AGAR POURED PLATES.—On peptone-beef agar the colonies become visible in about two days at room temperature and if well isolated reach a diameter of from 6 to 10 mm. in six days. The colonies are white or rather like boiled starch and translucent. After several days the color is greenish white. The bacteria in mass on a white background are yellowish green. The medium immediately surrounding the colonies becomes greenish, and this color eventually spreads over the whole plate. A single colony greens about one-fourth of an ordinary plate. The texture is at first soft and tender, and the colonies coalesce readily. If the plate is tipped, the colonies overflow the lower margin. Later the growth is thicker but not viscid. The colonies are circular, margins entire and definite, surface shining and smooth, with age occasionally slightly contoured, finally drying to a whitish film as transparent as the dry agar medium. A characteristic interior structure is the presence of numerous, tiny striae concentrically arranged. Sometimes these are coarser, more "fish-scale" in character. Only rarely have colonies occurred with "fish-scale" marks predominating (see Pl. 63, B). More often the "fish-scale" marks have been observed at the margin of colonies. This coarse marking rapidly changes into the finer lines termed "striae." The colonies which show these "fish-scale" markings are extremely thin and transparent. The striations usually disappear after six or seven days, though they have been observed in some cases in colonies 2 weeks old (Pl. 63, C). Oblique light is necessary for examination of these internal markings. In reflected or direct transmitted light, colonies often show a marginal band more opaque than the centers (Pl. 63, D). Buried colonies are dense and opaque and more or less irregular in shape.

The usual type of colony is best illustrated by Plate 63, A, E, shown under oblique lighting. Slight diversities in colony character seem to be caused by various factors such as moisture content of medium, degree of acidity or alkalinity of medium, temperature, length of time in artificial media, etc.

WHEY AGAR.—Formula: 1,000 cc. whey, 300 cc. water, 15 gm. agar flour, 15 gm. peptone, 15 gm. cane sugar, 3 gm. gelatin. Colonies smaller but thicker than on beef agar. At first almost hemispherical, translucent, and with pearly luster. The smooth surface becomes slightly contoured, and after four weeks the colony looks like a miniature rugged mountain. Buried colonies are translucent and larger than in beef agar, and the green stain is less conspicuous.

POTATO AGAR PLUS 1 PER CENT DEXTROSE.—The organism grows well on this medium, forming thick, almost opaque, greyish white colonies.

Striae, often coarse, sometimes even "fish-scale" in character, may be seen in the margins. A halo forms about the colonies. These halos are not cleared areas, but rather a whitish clouding fading gradually into the unchanged medium of the plate. In crowded plates this clouding so quickly coalesces that no halos are observed. After five or six days the colonies have a zoned appearance, the centers and borders being rather white, separated by a greyish zone. The internal marks are finer and more like those seen in beef agar.

AGAR STABS.—Surface growth only moderate, white, moist, and shining. Slight mesenteric growth in the upper part of the stab, but this does not persist. Old cultures show only surface growth. The whole medium becomes pale yellowish green ("Bright Chalcedony Yellow").[1]

AGAR STREAKS.—The growth is white, thin, transparent. The medium greens in 24 hours. The surface is very slightly contoured. The internal markings—striae—show along the margins for several days. On the upper part of the slant the bacteria are in a very thin layer, becoming thicker toward the lower part and with white sediment in the condensation water in the V.

GELATIN PLATES.—(+10 peptone-beef gelatin). In 48 hours at 20° C. the well-isolated colonies are 1½ mm. in diameter. The liquefied pits are shallow, saucer-shaped depressions, circular and with definite, entire margins. The liquid gelatin is slightly clouded, and the bacteria are mostly in a tiny white mass at the center of the depression. In 4 days well-isolated colonies are from 6 to 8 mm. in diameter. Thickly sown plates are entirely liquefied in 48 hours at 20°, with the colonies as tiny white masses floating in the slightly cloudy and slightly greened liquid.

GELATIN STABS.—Cultures kept at 17° to 18° C. have small liquefied pits on the surface in two days. The stab does not develop more than a trace of growth. The liquefaction becomes stratiform and proceeds slowly, being complete only after from five to seven weeks. At a temperature of 20° the liquefaction is more rapid, and the stab develops into a wide pocket containing numerous small white masses. At both temperatures the gelatin is greened.

BEEF-PEPTONE BOUILLON.—At room temperatures (20° to 27° C.) a slight clouding occurs at the surface in from 6 to 7 hours. The growth increases, always being best at the surface, forming clouds and pseudo-zoogloeae. In some cases a delicate pellicle forms. Rims are often present, thin, white, easily disintegrating. Growth is never very heavy in +10 to +14 beef bouillon. The slight sediment is white, fine-grained to flocculent, and is readily dissolved into clouds on shaking. Medium yellowish green ("Chalcedony Yellow" or "Chartreuse Yellow").

BEEF BOUILLON OVER CHLOROFORM.—Growth unrestrained.

[1] The colors mentioned in this paper are given according to Ridgway (RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.)

NEUTRAL, PEPTONE-BEEF BOUILLON.—Growth good, better than in + 10 beef bouillon. A pellicle forms. Medium greened.

LOEFFLER'S SOLIDIFIED BLOOD SERUM.—Only moderate growth and a very slow liquefaction with some strains of the organism. With one strain (No. 285) evidently no liquefaction.

POTATO CYLINDERS.—Growth at first dull, yellowish white, changing to a dirty, greenish brown ("Buffy Citrine"). The growth is moist and shining but not thick. The moister parts of the potato seem most favorable for growth. The diastatic action on the starch is moderate.

ACTION ON STARCH.—Potato cylinders on which the organism had grown for three weeks or more showed a moderate change in the starch. Tested with iodin the cultures gave reddish brown to purple reactions; the controls gave pure deep blue. This test was performed by crushing the cylinders and adding 10 cc. of water and 5 cc. saturated iodin in 40 per cent alcohol.

Peptone-beef agar plus 1 per cent potato starch was poured into Petri plates. When firm, the surface was inoculated by drawing a needle bearing bacteria across the center of the plate. Ten days later when the streaks were from 3 to 7 mm. wide the surface of the plates was flooded with iodin (saturated solution in 40 per cent alcohol). Most of the plate became intensely dark blue, except along the streak of bacterial growth where there appeared a clear area, or halo, from 2 to 6 mm. wide.

Under the microscope abundant starch particles could be seen in the agar, except in the halos where only a few scraps remained. A field in the halo gave by count only three tiny particles, while 10 cm. out in the blue agar a field of the same size gave over 200 large starch grains and many more small particles.

Plain peptone agar plus 1 per cent potato starch gave scantier growth to the bacteria, and the test with iodin showed no trace of halos.

Peptone-beef bouillon + 10 with 1 per cent potato starch.—Organisms were grown in this medium for 12 days. Then 2 cc. of iodin (saturated solution in 40 per cent alcohol) were added to the tubes. A reddish brown color developed, quickly and entirely fading out, while controls became and remained deep blue. Some of the same lot of cultures were tested with Fehling's solution. Reactions of cultures varied from orange to reddish purple; the controls were blue. After the cultures had settled for some hours a very slight reddish precipitate could be discerned in some of the tested tubes.

Uschinsky's solution plus starch.—A thin starch paste was added to Uschinsky's solution (about 20 cc. of starch paste to 100 cc. of the Uschinsky). Cultures grown in this for three and five weeks gave, when tested with iodin, brownish purple reactions. The controls were deep blue.

MILK.—Milk clears without coagulation. A watery band, rather greenish in color, begins at the surface and extends downward until the

whole tube is clear and translucent. The color is greenish yellow. With some strains this action is complete in five days, in others not until the twelfth day. Thin, white rims are present, no pellicles. The sediment is white, flocculent to curdy. In two months the color is light brown (between "Pinkish Buff" and "Cinnamon Buff") and the medium still translucent. No crystals are present.

LITMUS MILK.—In 24 hours the whole tube is slightly blued, and the surface shows a watery band of dark blue. This dark blue band deepens until in from 5 to 12 days the whole tube is very dark blue. In undisturbed cultures there may be seen three or more distinct bands of color, the darkest on the surface. In reflected light the color is almost black. There is no reduction. No further change was observed in two months. No crystals formed. In five different tests in litmus milk the results were uniformly as stated above. In a sixth test in a medium containing a smaller amount than usual of litmus, two strains (471 and 478) showed a trace of reduction on the tenth day. This did not increase, and on the fifteenth day the blue color was reestablished.

LITMUS AGARS WITH SUGARS.—On litmus-dextrose, litmus-saccharose, and litmus-galactose agar the medium was reddened within 24 hours. The acid action gradually increased for about 10 days then decreased, until after 5 weeks there remained almost no red color. One strain retained some red color in the dextrose and in the galactose media.

Litmus-lactose agar and litmus-glycerin agar never developed any red color. After five weeks the medium was a greenish blue, showing some change in the litmus.

Growth in these media was only moderate.

USCHINSKY'S SOLUTION.—A moderate to heavy growth is produced in this medium. Growth is best at the surface in the form of clouding, pseudozoogloeae, and a delicate pellicle. The medium becomes pale, apple green in color ("Veronese Green").

FERMI'S SOLUTION.—This gives an abundant growth. A delicate pellicle forms with clouding and pseudozoogloeae below. The color is pale bluish green ("Pale Veronese Green"). The pellicle becomes thicker and somewhat viscid. In two weeks it is about 2 mm. thick and difficult to break up.

COHN'S SOLUTION.—The organism does not grow in this medium.

TOLERATION OF SODIUM CHLORID.—Peptone-beef bouillons +13 containing 2, 3, 4, and 5 per cent of sodium chlorid were inoculated from young agar cultures. Growth occurred in all but was rather scanty in the 5 per cent. The experiment was repeated using 4, 5, and 6 per cent of sodium chlorid in +13 beef bouillon. Again growth was scanty in the 5 per cent. No growth occurred in the 6 per cent.

FERMENTATION TUBES.—The medium used was 2 per cent Witte's peptone and 1 per cent, respectively, of each of the following: dextrose, lactose, saccharose, maltose, mannit, glycerin, and levulose. The open

end of the tubes clouded in from 2 to 3 days, and in two weeks the growth was moderate to heavy. There was a very faint clouding in the closed ends with mannit, saccharose, and levulose. In the dextrose tubes the closed end seemed to be cloudy at first, but at the end of two weeks these were entirely like the controls. No gas formed in the closed arm in any media. When tested with litmus paper on the twentieth day the dextrose cultures gave an acid reaction, glycerin cultures were neutral, and the others—mannit, saccharose, maltose, lactose, and levulose—were alkaline. The control tubes were acid.

NITRATES ARE NOT REDUCED.—Growth in the nitrate-beef bouillon is good, and a pellicle forms. Cultures were tested when 10, 17, and 24 days old. Starch water, potassium-iodid solution, and sulphuric acid were used in testing for nitrite. No change of color occurred in any of the cultures—that is, no nitrite is formed.

HYDROGEN SULPHID is produced in small quantities as determined by the slight browning of lead-acetate paper suspended over cultures growing in beef bouillon, beef agar, and in milk.

INDOL is produced in small but definite amounts in the following medium: 1 per cent peptone, 0.5 per cent disodium phosphate, and 0.1 per cent magnesium sulphate in distilled water. Cultures in 1 and 2 per cent peptone (Witte's), Dunham's solution, and Uschinsky formed no demonstrable amounts of indol.

AMMONIA production is feeble. Beef bouillon cultures were tested with Nessler's solution. Strips of filter paper moistened in the solution were suspended over the cultures, which were then heated. A brownish color developed on the paper. A second test was made, using filter paper colored with haematoxylin. This changed from a reddish color to purple in the heated culture tubes.

TOLERATION OF ACIDS.—The toleration of acids was tested in +10 peptone-beef bouillon to which were added different percentages of citric, malic, tartaric, and hydrochloric acids. Sufficient acid was added to the bouillon to make 0.05, 0.10, 0.15, and 0.20 per cent solutions. One cc. of 0.5, 1, 1.5, and 2 per cent acid solutions, respectively, were added to 9 cc. of the +10 bouillon. The acid was added under sterile conditions and the solutions used four days later without heating. The organisms grew well in the 0.05 and the 0.10 per cent solutions of citric, malic, and tartaric acids. Growth was good within 24 hours. Heavy pellicles formed and the media became green. One strain (No. 471) grew in the 0.15 per cent solutions of citric, malic, and tartaric acids. All strains grew in the 0.15 per cent solution of hydrochloric acid. None grew in a 0.15 per cent solution of oxalic acid. No growth was obtained in any of the 0.20 per cent solutions with any of the strains of the organism.

TEMPERATURE RELATIONS.—In +10 peptone-beef bouillon the optimum temperature is 25° to 28° C. The maximum is between 36° and 37°,

and the minimum is below 2°. The thermal death point is between 48° and 49°.

DESICCATION.—Bacteria from 24-hour beef bouillon cultures, spread on sterile cover glasses and kept in the dark, were nearly all dead inside of 24 hours. A few grew after 7 days' drying.

Bacteria from 7-day-old agar cultures showed more resistance and were alive on the ninth day after being put on covers. A few were alive on the twelfth day, and one culture was secured after 26 days. The organism has been isolated from dry wheat kernels kept at room temperature for 17 months.

OPTIMUM REACTION IN BEEF BOUILLON.—The organism grows well in peptonized beef bouillons titrating from 0 to −14 on the alkali side and to +30 on the acid side. Beyond these limits the growth was feeble or lacking. Sodium hydrate was used for the alkali and hydrochloric for the acid. These were inoculated rather heavily from 2-day-old beef bouillon cultures.

EFFECT OF SUNLIGHT.—Five minutes' exposure to sunlight, on ice, killed about 50 per cent of the organisms in agar plates. Ten minutes' exposure caused a reduction of from 93 to 100 per cent.

EFFECT OF FREEZING.—Several tests were made. (1) Freshly inoculated beef bouillon cultures were frozen for two hours. The reduction of the organism was 70 to 75 per cent. (2) Five hours' freezing caused a reduction of from 84 to 100 per cent. (3) Forty-four hours' freezing killed 80 to 100 per cent.

NAME OF ORGANISM

This organism appears to be an undescribed form, and because of the brown to black discolorations which it causes at the base of the wheat kernels and glumes the name *Bacterium atrofaciens*, n. sp., is suggested.

TECHNICAL DESCRIPTION

Bacterium atrofaciens, n. sp.[1]

Rods, cylindrical, rounded at ends. Individual rods 1 to 2.7μ by 0.6μ. In liquid media, moderately long filaments (3 to 30 bacteria). Motile by 1 to 4 polar flagella; aerobic, no spores; capsules in beef agar cultures.

Surface colonies in peptone-beef agar plates are round, smooth, shining, and with inconspicuous internal striations, somewhat irregularly concentric in arrangement, white becoming greenish, the surrounding medium also becoming greenish.

[1] *Bacterium atrofaciens*, sp. nov.

Baculis asporis, aerobiis. Capsulas in culturis agar-agar habentibus. Baculis singularibus 1-2.7×0.6μ. 1-4 flagellis polaribus mobilibus. In culturis liquidis 3-30 baculis in filamentis conjunctis. Coloniis albis, rotundis, nitentibus, et in agar-agar cum striis inconspicuis internis, aliquid irregulariter concentricis; Culturae et coloniae virescentes.

Gelatinam liquefacit. Nitrum non redigitur. Lac sterile alcalinum fit, non coagulatur. Saccharum saccari, saccharum uvae, et saccharum galacti acidum fit; gas non fit. Baculi methodo Gram non colorantur.

Habitat in foliis, glumis, et granis *Tritici vulgaris*. Maculas fuscas in basi glumarum granorumque facit.

Gelatin is liquified; milk becomes alkaline and translucent but does not coagulate; nitrates not reduced; acids produced from saccharose, dextrose, and galactose. No gas produced. Gram-negative. Group number 221.2322123.

Pathogenic to *Triticum vulgare*, causing especially discoloration and lesions at the base of glumes and kernels.

INOCULATIONS

Inoculations on young wheat plants have given numerous leaf infections (Pl. 62, D). Fewer mature plants have been available for inoculation of heads, and weather conditions were rather unfavorable; but enough infections were secured to determine the positive pathogenicity of the organism for glumes (Pl. 62, E, F) and kernels.

For the leaf inoculations bacteria from agar subcultures were mixed with sterile water and sprayed on the plants with an atomizer. The infections are stomatal. On the second day after the inoculation, dark, water-soaked spots appear. These are small and well distributed over the leaf surface. Two days later the spots are pale yellow. They enlarge and also elongate slightly. Later the color is light brown and the tissues are dry.

Sometimes bacteria were added directly to drops of condensation water on the leaf tips. In these inoculations the whole tip of the leaf became yellowish brown and shriveled. If examined at the end of five or six days the bacteria are abundant in the tissues. From the leaf infections (more than 50 series of successful inoculations) the organism was reisolated 22 times and often used again, producing typical infections.

For inoculating the heads of wheat, bacteria from agar subcultures were diluted in sterile water and by means of a camel's hair brush were spread over the glumes and between the spikelets of young heads just emerging from the flag leaf. On some glumes a few slight wounds were made with a fine needle.

The infections (all done in Washington, D. C.) were slower in showing on the heads than on the leaves. Glume discolorations were noted first on the fourth day after inoculation. The spots enlarged slowly and never became so noticeable as those on naturally infected heads. Examination after collection showed that the bacteria had penetrated to and into the kernels, some of which showed the characteristic blackening at the germ end and great abundance of bacteria in the spaces about the radicle. The inner surface of the glumes also showed more discoloration than was visible from the exterior. (See Pl. 62, F, *a*.)

The characteristic organism was reisolated from both the glumes and the kernels of the inoculated heads and used again in a second series of inoculations on heads and on leaves of seedling plants. The disease was reproduced in both series, and the organisms were again isolated from each

SUMMARY

A bacterial disease of wheat caused by a hitherto undescribed organism has been found on heads of wheat collected in various and widely separated localities in the United States and Canada.

The most noticeable external character of the disease is the brown to black discoloration on the lower part of the glumes and of the adjacent rachis. The grains inclosed by the diseased glumes have bacteria in the tissues at the germ end. In advanced cases this end of the grain is black and charred in appearance.

The discolored tissues swarm with bacteria. These have been isolated not only from freshly collected material but also from grain kept in the laboratory for 17 months.

The parasite is a white, polar-flagellated rod, producing a green fluorescence in the ordinary culture media.

The group number is 221.2322123.

Bacterium atrofaciens, n. sp., is suggested as the name.

PLATE 62

Basal glumerot of wheat:

A.—Heads showing diseased glumes. Collected June, 1917. Kansas. Photographed May, 1918. ×1½.

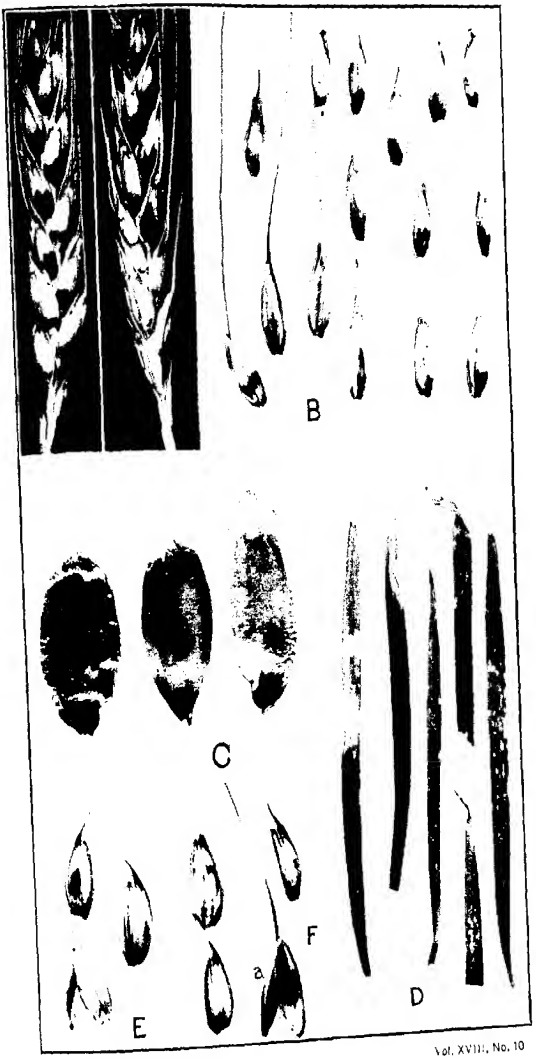
B.—Glumes diseased at base. Collected in New York and Alberta, Canada. ×1½.

C.—Grains black and full of bacteria at base. From New York and Canada. ×6.

D.—Young wheat leaves five days after inoculation with *Bacterium atrofaciens*.

E.—Glumes inoculated with strain 478. May, 1918.

F.—Glumes inoculated with strain 399. *a*, Inner face. May, 1918.


B
C
D
E
F
a

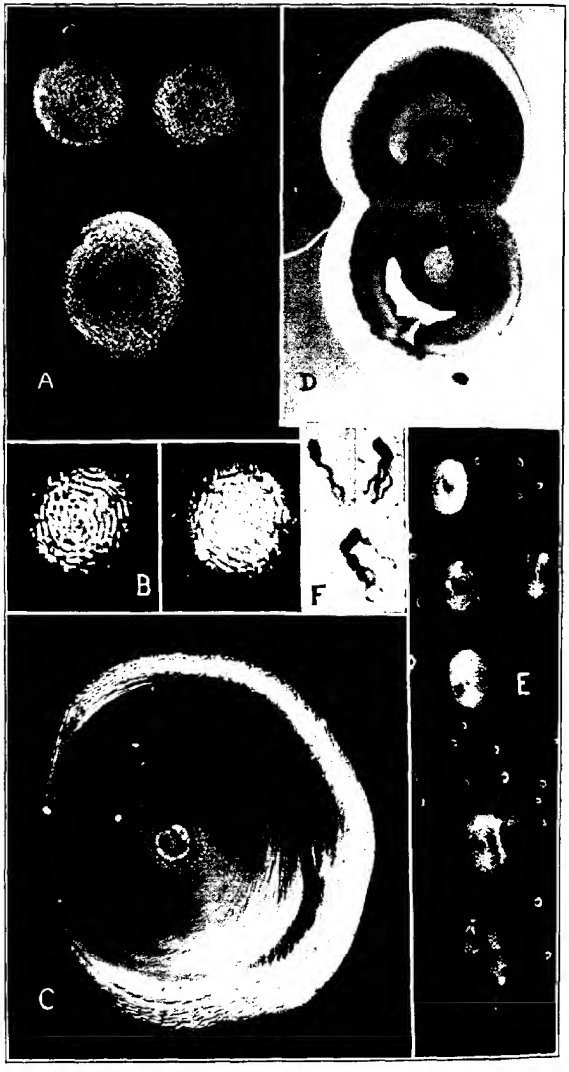

A
D
B
F
E
C

PLATE 63

Various types of colonies of *Bacterium atrofaciens:*

A.—Moderately crowded colonies on agar plate, 3 days old. Oblique light. ×10.

B.—Well-isolated colonies on agar plate, 2 days old. Oblique transmitted light. ×10.

C.—Single colony on agar plate, 20 days old. Oblique transmitted light. ×6.

D.—Well-isolated colonies on agar plate, 3 days old. Direct transmitted light. ×10.

E. Crowded colonies on agar plate, 2 days old. Oblique transmitted light. ×10.

A and E represent the ordinary type of young colony. B and D are atypical.

F.—Flagella (Van Ermengem's stain). Stained by M. K. Bryan. Photographed by Erwin F. Smith. About ×2,000.

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